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Bio–based blends of achira starch and polyvinyl alcohol: mechanical and thermal approach

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ABSTRACT

This work aimed to study thermo-mechanical behavior of blends based on polyvinyl alcohol (PVA), achira starch and glycerol as plasticizer. Several formulations were prepared using an experimental mixture design. Initially, raw materials were mixed in a roll mill at 60°C. Afterwards, these blends were molded at 110°C and 38 MPa to obtain translucent and flexible films. Tensile, scanning electronic microscopy (SEM), differential scanning calorimetry, thermo gravimetric and Fourier transform infrared (FTIR) analysis were conducted to evaluate the interaction among components. Mechanical results indicated that deformation mechanism of studied blends is probably ascribed not only to rearrangement of PVA microstructure, but also to starch granules characteristics. Besides, SEM micrographs corroborated the strong influence of shape and size range granules on the fracture mechanism. Moreover, the addition of starch, PVA and plasticizer increased Young's modulus, tensile strength and elongation at break, respectively. Thermal evaluation suggested a slightly influence of starch gelatinization on enthalpy of endothermal process as well as a correlation between mass loss fraction and second order interactions. Likewise, FTIR data let take more certain about the presence of inter- and intra-molecular hydrogen bonds among components of the blend.

Keywords—*PVA/starch blend, thermoplastic starch, polyvinyl alcohol, glycerol*

I. INTRODUCTION

During the last decade, there have been intensive attempts to develop bio-based/nonbiodegradable and biodegradable polymers with synthetic and natural raw materials. The growth in the use of biopolymers includes especially packaging (flexible & rigid) and textile market segments. Thus, international projections estimate that the global production capacity of those materials will rise around 2.4 million tons in 2022 [1].

Starch, a naturally occurring polymer, has been considered as a viable alternative in developing biodegradable polymers due to its relative abundant availability and low cost. The approaches toward promising starch-based biopolymers entail to overcome disadvantages mainly related to its strong hydrophilic nature and poor mechanical integrity [2]. It was found a progressive development of those materials through chemical modifications of starch, thermoplastic starch [3] and blends of starch with other biopolymers such as polylactic acid, polybutylene succinate, poly(3-hydroxibutirate) and polyvinyl alcohol (PVA) [4][5].

In particular, the hydrophilic nature of PVA promotes compatibility with other biodegradable materials by providing a stable supporting surroundings to the formulation of thermoplastic materials [6]. Additionally, PVA is a biodegradable material wide use due to its versatility, barrier properties and low

toxicity [7]. In this sense, PVA/starch blend is well suited to develop biopolymers with applications in both packaging and agricultural sectors [8].

The plasticizers are strongly necessary to obtain moldable thermoplastic films of PVA/starch blend; therefore, several substances have been studied such as sorbitol, polyethylene glycol, 1,4-butanediol, pentaerythritol, 1,2,6-hexanetriol, xylitol, mannitol, sucrose, urea, ascorbic acid, citric acid, succinic acid, malic acid, tartaric acid and so on [2][9]. Nevertheless, glycerol (1,2,3-propanetriol) is a suitable plasticizer to attain homogeneity, flexibility and workability of the films [10][11].

Although PVA and traditional starches blends have been extensively investigated, further comprehensive analysis of using alternative starches is still needed. Based on this perspective, this work aims to use the achira (*Canna edulis*) starch as a raw material to formulate PVA/starch blends with glycerol as plasticizer. The effect of different concentrations of components on the mechanical and thermal behavior were investigated.

II. Materials and Methods

A. Materials

Native achira starch (food grade) was purchased from CAMARI-FONDO ECUATORIANO POPULORUM PROGRESSIO (Quito, Ecuador). PVA 88% hydrolysis degree was supplied by SEKISUI CHEMICAL CO., LTD (Osaka, Japan). Zinc stearate was acquired from SIGMA-ALDRICH (Darmstadt, Germany). Glycerol (99% purity) was provided by LOBA CHEMIE PVT. LTD (Mumbai, India); this plasticizer was used without further purification.

B. Blends preparation

PVA and achira starch were dried in an oven MMM Group VENTICELL, model LSIS-B2V/VC 55 (Munich, Germany) for 48 hours at 50°C to remove moisture. In order to formulate blends based on starch, PVA and glycerol, several formulations were prepared using an experimental mixture design with lineal constraints. On the upper and lower limits PVA:starch ratios were 70:30 and 50:50, respectively. The percentage range selected for plasticizer was 15 wt. % to 35 wt. %. The Minitab 1.7 software was used to obtain several formulations statistically representative. In consequence, thirteen formulations were prepared according to the composition shown in Table I and plotted in Figure 1. The codification GXPY was used to identify each formulation, according to the following description: G (glycerol), X (glycerol wt. % over the formulation), P (ratio PVA:starch) and Y (PVA wt. %/ starch wt. %).

TABLE I. COMPOSITION OF BLENDS*

Sample	Glycerol	PVA	Starch
G15P1	0.150	0.423	0.423
G15P1.5	0.150	0.507	0.338
G15P2.3	0.150	0.592	0.254
G20P1.2	0.200	0.435	0.360
G20P1.9	0.200	0.519	0.276
G25P1	0.250	0.373	0.373
G25P1.5	0.250	0.447	0.298
G25P2.3	0.250	0.522	0.224
G30P1.2	0.300	0.385	0.310
G30P1.8	0.300	0.449	0.246
G35P1	0.350	0.323	0.323
G35P1.5	0.350	0.387	0.258
G35P2.3	0.350	0.452	0.194

*Remaining fraction corresponds to zinc stearate

To process each formulation, raw materials were mixed in a bench-type roll mill COLLIN model W 100 T (Ebersberg, Germany) at 60°C and 80 rpm. Afterwards, these blends were molded using a laboratory hydraulic press CARVER, model 2112 (Menomonee, USA) for 7 min at 110°C under constant pressure of 38MPa.

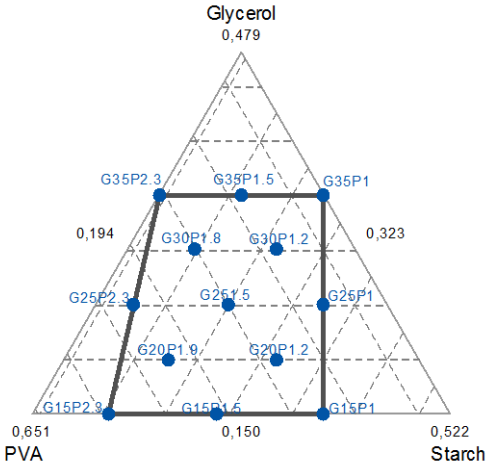

Fig. 1. Ternary plot of studied blends

C. Mechanical assessment

Young modulus, tensile strength and elongation at break of thirteen formulations were determined by using universal testing machine INSTRON model 3365 (Norwood, USA) at room temperature according to ASTM D638-14 standard. The test was carried out at a crosshead speed of 50 mm/min. At least five samples were tested for each formulation.

Detailed inspection of fracture surfaces on broken specimens was performed by means of scanning electron microscope TESCAN model VEGA II LMU (Brno, Czech Republic) at an accelerating voltage of 15 kV.

D. Thermal evaluation

Differential scanning calorimetry (DSC) evaluation was recorded using a calorimeter NETZSCH 204, model F1 Phoenix (Munich, Germany). Samples were evaluated according to standard method ASTM D3418-15 from -20°C to 250°C at 20°C/min.

In addition, thermogravimetric analysis (TGA) was performed by a thermo-balance SHIMADZU, model 50 (Kyoto, Japan). The analysis was conducted according to ASTM E1131-8(2014) standard. Measurements were tested from 23°C to 650°C at 3°C/min, under nitrogen atmosphere with the flow rate of 50 cm3/min.

E. FTIR analysis

Infrared spectra of pristine materials and PVA/starch films were accomplished in attenuated total reflectance mode on a spectrophotometer PERKIN ELMER, model spectrum one (Beaconsfield, United Kingdom). The spectra were obtained from 650 cm^{-1} to 4000 cm^{-1} at a resolution of 4 cm^{-1}.

III. Results and Discussion

A. Mechanical behavior

From Figure 2 can be observed that PVA/starch blends are translucent and homogeneous on a macroscopic scale. Nevertheless, specimens evidence optical differences after tensile test which are associated to deformation mechanisms in the plastic flow regime. In particular, under uniaxial stretching PVA probably undergo a chain-folded spherulite rearrangement through a crystallographic slip mechanism within the lamellae [12].

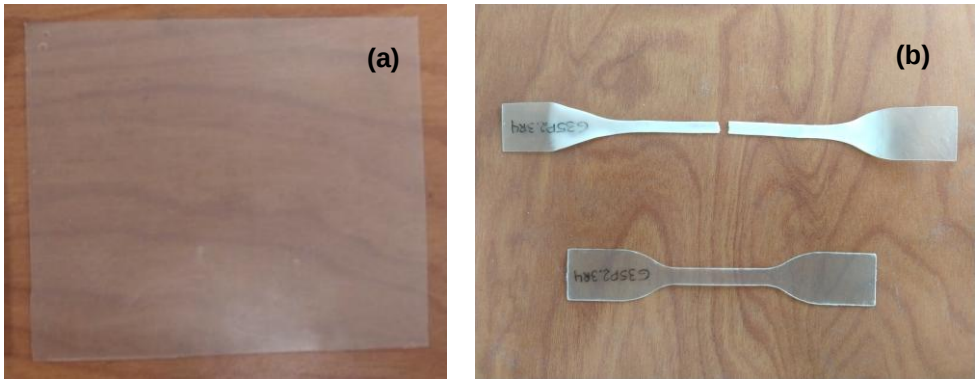

Fig. 2. Visual aspect of (a) PVA/starch film and (b) specimen before and after the tensile test

Figure 3 presents the stress-strain curves obtained for different contents of raw materials. All samples exhibit elastic regime at low deformations and plastic area at large strain values. Mechanical behavior of PVA/starch films is characterized by the absence of an overshoot typical of yield-stress semicrystalline polymers. However, curves suggest that elastic zone is followed by a deformation of crystalline domains of PVA. Moreover, high values of elongation indicate that recrystallization process are also possible.

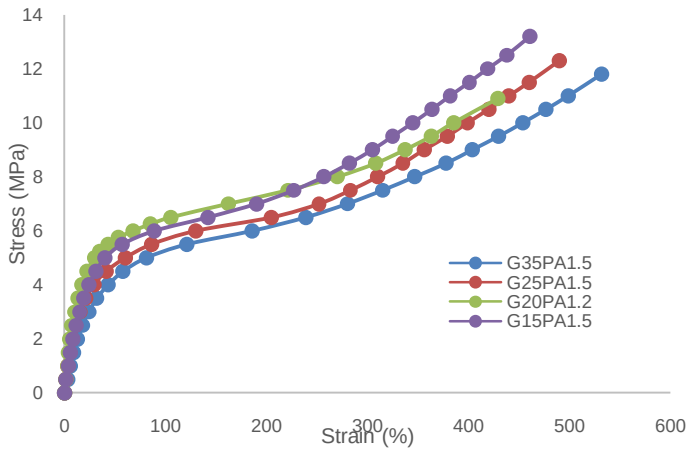

Fig. 3. Tensile curves of PVA/starch blends

Tensile properties of evaluated specimens are shown in Table II. Initially, it is noticed that samples with high proportion of starch exhibit dispersion values higher than the other formulations. This variability is possibly correlated not only with starch granule size but also with differences in the cohesion forces among components. The strong interchain interactions of blend components, through hydrogen bonds, produce some difficulty in the shift of polymer chains during the mechanical test.

The influence of component contents is presented in Figure 4. From contour plots patterns, it can be observed a directly proportional relation between Young modulus and achira starch; therefore, the contribution of starch to elastic behavior of blends is minimal, in accordance with similar observations for PVA/starch blends [13]. Furthermore, the addition of PVA and glycerol increase tensile strength and elongation at the break, respectively.

TABLE II. MECHANICAL PROPERTIES OF PVA/STARCH BLEND FILMS UNDER TENSILE EVALUATION

Sample	Young modulus (MPa)	Tensile strength(MPa)	Elongation at the break (%)
G15P1	45,76 ± 0,93	8,66 ± 0,59	416 ± 14
G15P1.5	26,86 ± 0,88	11,06 ± 0,48	472 ± 27
G15P2.3	17,62 ± 0,72	13,24 ± 0,41	483 ± 25
G20P1.2	21,20 ± 0,91	9,25 ± 0,58	516 ± 29
G20P1.9	16,64 ± 0,70	11,90 ± 0,78	558 ± 17
G25P1	19,10 ± 0,54	7,61 ± 0,59	518 ± 22
G25P1.5	12,92 ± 0,64	9,48 ± 0,48	607 ± 19
G25P2.3	7,45 ± 0,19	13,24 ± 0,43	654 ± 23
G30P1.2	10,55 ± 0,27	8,03 ± 0,44	602 ± 15
G30P1.8	7,67 ± 0,51	10,16 ± 0,33	678 ± 22
G35P1	7,34 ± 0,41	5,83 ± 0,72	588 ± 25
G35P1.5	5,28 ± 0,91	8,10 ± 0,27	631 ± 18
G35P2.3	4,94 ± 0,20	11,76 ± 0,39	695 ± 20

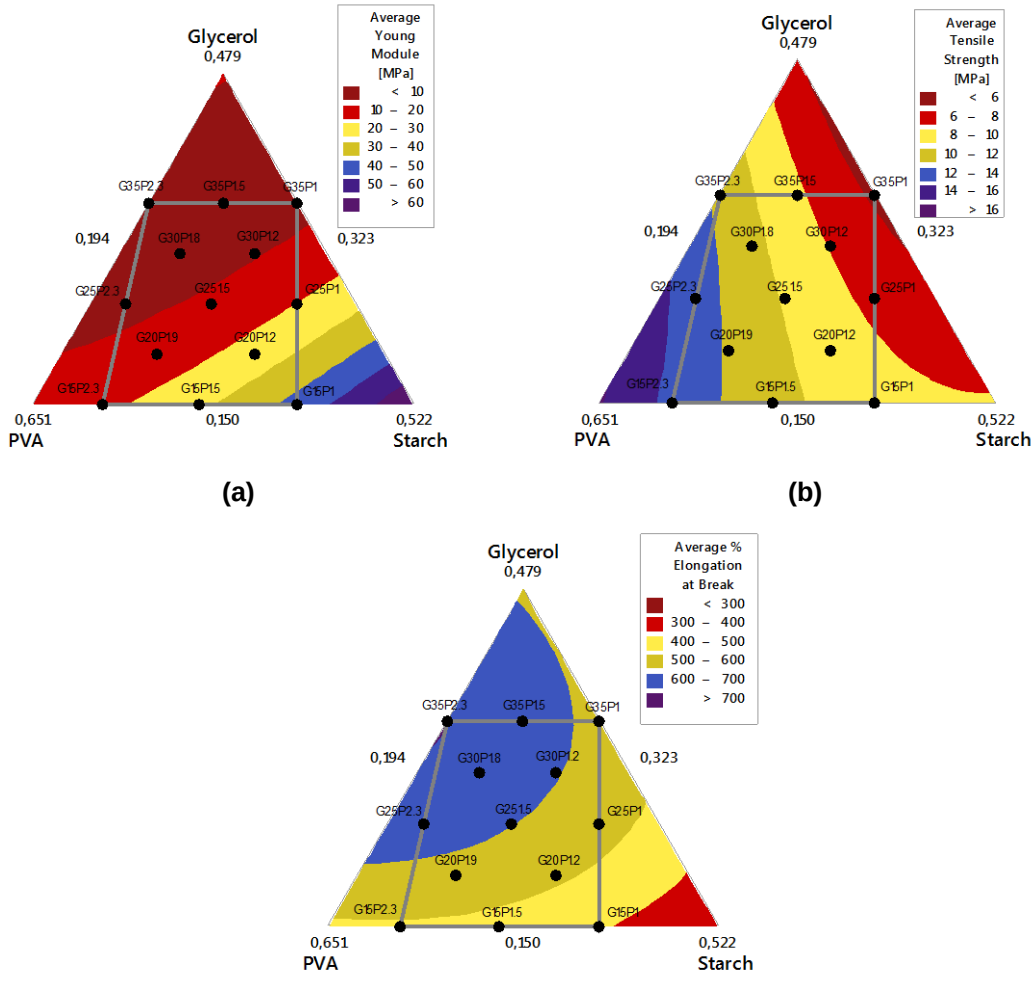

Fig. 4. Contour plot of (a) Young modulus (b) tensile strength and (c) elongation at break

SEM micrographs of Figure 5 show numerous undestroyed starch granules on fracture surface suggesting that mixing and molding process did not affect the totality of granules. Microstructural surface describes a dimple like geometry which is a characteristic of ductile fracture mechanism. An analysis within a mechanical fracture context suggests that critical defects are related with starch microstructure. Moreover, micro-voids were apparently originated not only for oval-shaped but also due to the size range of granules. In terms of granules distributions of achira starch, previous investigations

reported a wide range of granule size [14], [15]; that disparity leads to numerous stress concentration sites which affect the fracture behavior of studied blends. From the perspective of fracture energy on the defect's presence, fracture probably has occurred by stress values lower than theoretical cohesive resistance.

It is worth noting that, mechanical behavior is not exclusively associated with starch granules characteristics. Elastic and plastic deformation as well as final fracture surface are also influenced by the reorientation of crystalline zones in the direction of applied remote tensile and destruction of some of the largest crystalline domains [12].

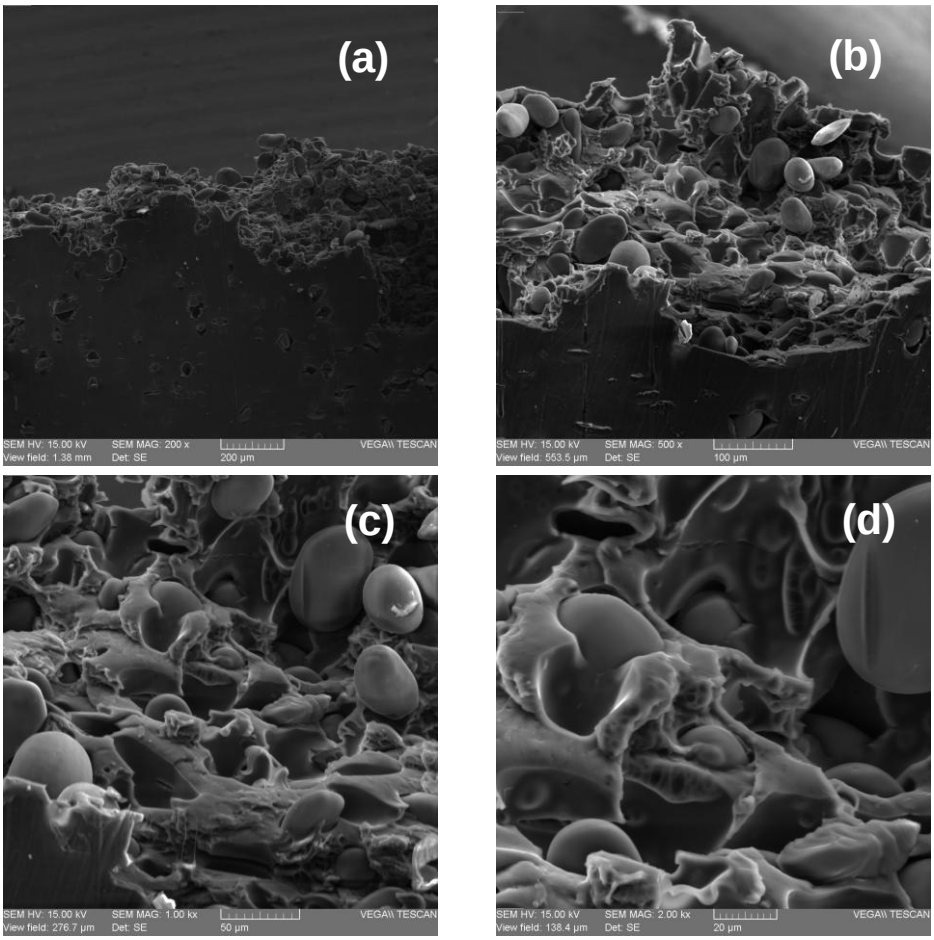

Fig. 5. SEM images showing fractographic features of PVA/starch blend films (a) 200X (b) 500X (c) 1000X and (d) 2000X

B. DSC evaluation

DSC measurements summary of PVA/starch blend films are presented in Figure 6. The enthalpy of melting process was extracted from thermograms and plotted in Figure 7. It can be observed in Figure 6 that there are no discernible changes of the thermal behavior of samples. DSC traces of all specimens show an overlap of some events spread over a large temperature range (20–140ºC). These endothermal transitions have been discussed extensively; however, thermal behavior of PVA/starch blends in this range is not well understood. Thermal events shown in the present study may hide glass transition temperature (Tg) due to partially crystalline starch microstructure, amorphous chains surrounded by crystalline zones and water release, in agreement with other authors [2][16][17]. Additionally, hydrogen bonding produced not only between hydroxyl groups of PVA and starch but also among functional groups of PVA, starch and plasticizer suppose a higher chain rigidity. Therefore, these strong interactions through inter- and intra-molecular hydrogen bonds may influence on the water release and consequently on identification of thermal transitions patterns corresponding to Tg.

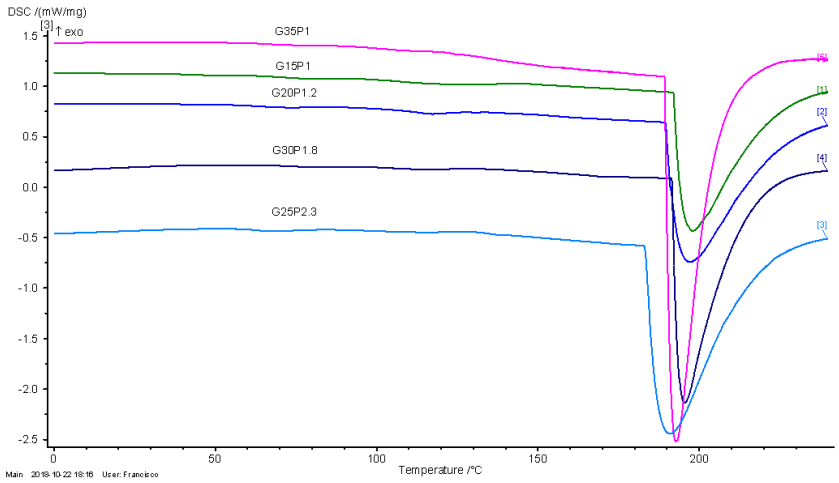

Fig. 6. DSC scans for PVA/starch blends

On the other hand, a broad endothermal peak was detected from 180ºC to 250ºC, which is attributed to the melting of the crystal of PVA. In this case, the variations of enthalpy of this thermal event are presumably related to the effect of plasticizer on the formation of PVA crystallites due to hydrogen bonds. Glycerol molecules size avoid an easily accommodation in the crystal lattice of PVA leading to smaller crystallites and defectives in the lamellar morphology [17]. In addition, undestroyed granules observed on SEM micrographs suggest that enthalpy of endothermal peak is slightly affected by starch gelatinization. Thus, the probability of stronger hydrogen bonding resulting from starch gelatinized with PVA is reduced [11].

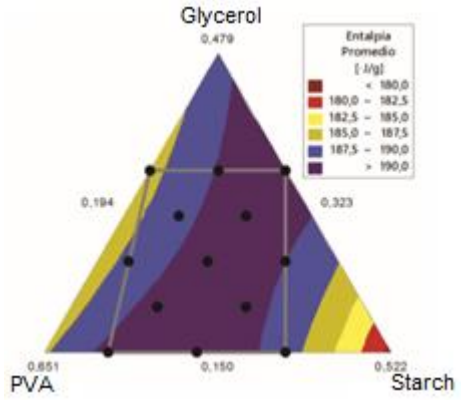

Fig. 7. Contour plot of endothermal enthalpy in the range of 180 ºC to 250ºC

C. Thermal stability

Thermogravimetric data listed in Table III indicate four decomposition stages in the tested range. The first step is associated to evaporation of free water and to the beginning of volatiles loss. Second thermal decomposition is related to the evaporation process of glycerol and bounds water. However, during this step it is also produced the evaporation of water which is generated by the detachment of hydroxyl groups of PVA and their reaction with the hydrogen of the PVA main chain [10][18][19]. The third stage, with the highest degradation rate, is probably ascribed to the PVA and starch nonoxidative processes generating carbon monoxide, carbon dioxide, acetaldehyde and acetic acid [17]. Last phase is attributed to decomposition of low molecular weight structures which were produced by PVA in previous stages. It should be noted that all formulations exhibit similar thermogravimetric behavior in terms of total mass loss. Likewise, the greatest thermal decomposition takes place above 245ºC. This pointed out a possible correlation between mass loss fraction and interaction among components through hydrogen bonds [20][17].

TABLE III. THERMOGRAVIMETRIC CHARACTERISTICS OF PRISTINE COMPONENTS AND PVA/STARCH BLENDS

Sample	Mass loss in each decomposition stage (%)				Total mass loss (%)
	First	*Second*	*Third*	*Fourth*	
	23–170°C	*170–245°C*	*245–390°C*	*390–800°C*	
PVA	4.55	59.07		28.77	92.39
Starch	15.65	53.60		-	69.25
Glycerol	32.49	-	-	-	32.49
G15P1	9.06	11.75	47.97	10.04	78.82
G15P1.5	9.14	11.76	47.93	10.25	79.08
G15P2.3	9.13	11.77	47.25	10.01	78.16
G20P1.2	9.97	13.04	45.10	10.37	78.48
G20P1.9	10.25	11.73	45.15	11.23	78.36
G25P1	9.86	15.57	43.68	9.52	78.63
G25P1.5	10.87	13.69	41.78	9.75	76.09
G25P2.3	10.18	14.54	43.79	10.31	78.82
G30P1.2	10.55	12.82	38.86	10.58	72.81
G30P1.8	11.87	14.98	38.20	7.69	72.74
G35P1	11.74	17.25	37.18	8.26	74.43
G35P1.5	11.12	13.54	37.58	9.66	71.90
G35P2.3	11.71	18.26	36.15	10.76	76.88

D. FTIR analysis

Figure 8 shows a summary of FTIR spectra of studied materials whereas Table IV presents main changes among spectra of raw materials and PVA/starch blends, in terms of frequencies of relevant functional groups. In general terms, it has been identified a number of different changes in all analyzed vibrational groups.

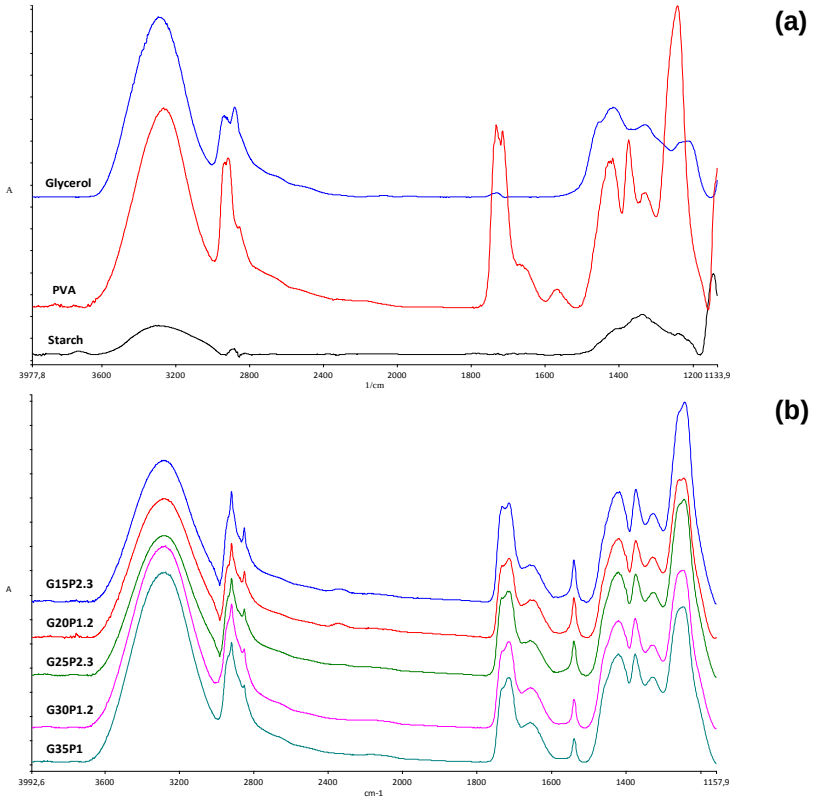

Fig. 8. FTIR spectra of (a) pristine componentsand (b) PVA/starch blends

TABLE IV. SUMMARY OF FTIR RESULTS

Sample	Functional group			
	OH stretching	*Bound water*	*C–O stretching of C–O–H*	*C–O stretching of C–O–C*
PVA	3265	1650	-	1086
Starch	3310	-	1144	1077
Glycerol	3294	-	1107	-
G15P1	3292	1657	1141	1026
G15P1.5				
G15P2.3				
G20P1.2	3279	1655	1142	1089
G20P1.9				
G25P1	3279	1655	1141	1090
G25P1.5				
G25P2.3				
G30P1.2	3273	1655	1141	1092
G30P1.8				
G35P1	3272	1655	1141	1094
G35P1.5				
G35P2.3				

PVA exhibit a band at 3265 cm^{-1} which is ascribed to stretching vibration of free and bonded OH groups. Blends show absorption bands in the range of 3272 cm^{-1} to 3292 cm^{-1} showing an evident shift to higher wave number [21]; this behavior implies an increase of intermolecular hydrogen bonding. Based on the starch OH stretching point of view, a substantial movement to lower frequencies is also observed [22].

The band in 1660 cm^{-1} corresponding to deformation vibration of the OH groups increase to a higher frequency region by around 5-7 cm−1 suggesting a strong hydrogen bond among components. In addition, C–O stretching of C–O–H functional group, detected in 1144 cm^{-1} in starch, slightly shift to lower frequencies (1141-1142 cm^{-1}) which is probably related to variation of hydrogen bonding ratio in the PVA/starch blends. In addition, changes in C–O stretching of C–O–C band suggest a possible transform into double peaks due to the effect of glycerol in starch microstructure [2]. Therefore, FTIR changes of vibrational functional groups corroborate the presence of inter- and intra-molecular hydrogen bonds among blend components argued in mechanical and thermal analysis.

IV. CONCLUSION

PVA/starch blends, based on an experimental mixture design, were formulated successfully. Mechanical results indicated that deformation mechanism of studied blends is probably associated to chain-folded spherulite rearrangement of PVA as well as to starch granules characteristics. The fracture surface corroborates the strong influence of shape and size range granules on the formation of critical defects which in turn lead to numerous stress concentration sites. From the perspective of mechanical properties, the addition of starch, PVA and plasticizer increase Young modulus, tensile strength and elongation at the break, respectively.

Both SEM and DSC evaluation evidenced a slightly influence of starch gelatinization on enthalpy of endothermal process. According to thermogravimetric analysis, the greatest thermal decomposition took place above 245ºC suggesting a correlation between mass loss fraction and second order interaction among components. Reported FTIR data, indicate the presence of inter- and intra-molecular hydrogen bonds which could promote the compatibility among blend components and consequently improve mechanical and thermal integrity.

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REFERENCES

[1] European Bioplastics and Institute for Bioplastics and Biocomposites, "Report - bioplastic marked data 2017," *Eur. Bioplastics*, pp. 1–7, 2017.

[2] A. A. Aydin and V. Ilberg, "Effect of different polyol-based plasticizers on thermal properties of polyvinyl alcohol:starch blends," *Carbohydr. Polym.*, vol. 136, pp. 441–448, 2016.

[3] X. L. Wang, K. K. Yang, and Y. Z. Wang, "Properties of starch blends with biodegradable polymers," *J. Macromol. Sci. - Polym. Rev.*, vol. 43, no. 3, pp. 385–409, 2003.

[4] S. Do Yoon, M. H. Park, and H. S. Byun, "Mechanical and water barrier properties of starch/PVA composite films by adding nano-sized poly(methyl methacrylate-co-acrylamide) particles," *Carbohydr. Polym.*, vol. 87, no. 1, pp. 676–686, 2012.

[5] W. L. Chai, J. D. Chow, C. C. Chen, F. S. Chuang, and W. C. Lu, "Evaluation of the biodegradability of polyvinyl alcohol/starch blends: A methodological comparison of environmentally friendly materials," *J. Polym. Environ.*, vol. 17, no. 2, pp. 71–82, 2009.

[6] N. Rescignano *et al.*, "PVA bio-nanocomposites: A new take-off using cellulose nanocrystals and PLGA nanoparticles," *Carbohydr. Polym.*, vol. 99, pp. 47–58, 2014.

[7] R. Jayasekara, I. Harding, I. Bowater, G. B. Y. Christie, and G. T. Lonergan, "Preparation, surface modification and characterisation of solution cast starch PVA blended films," *Polym. Test.*, vol. 23, no. 1, pp. 17–27, 2004.

[8] A. Akhavan, F. Khoylou, and E. Ataeivarjovi, "Preparation and characterization of gamma irradiated Starch/PVA/ZnO nanocomposite films," *Radiat. Phys. Chem.*, vol. 138, no. May 2016, pp. 49–53, 2017.

[9] X. Y. Zhou, Y. F. Cui, D. M. Jia, and D. Xie, "Effect of a complex plasticizer on the structure and properties of the thermoplastic PVA/starch blends," *Polym. - Plast. Technol. Eng.*, vol. 48, no. 5, pp. 489–495, 2009.

[10] B. Sreedhar, D. K. Chattopadhyay, M. S. H. Karunakar, and A. R. K. Sastry, "Thermal and surface characterization of plasticized starch polyvinyl alcohol blends crosslinked with epichlorohydrin," *J. Appl. Polym. Sci.*, vol. 101, no. 1, pp. 25–34, 2006.

[11] X. Luo, J. Li, and X. Lin, "Effect of gelatinization and additives on morphology and thermal behavior of corn starch/PVA blend films," *Carbohydr. Polym.*, vol. 90, no. 4, pp. 1595–1600, Nov. 2012.

[12] S. Jabbari-Farouji, J. Rottler, O. Lame, A. Makke, M. Perez, and J. L. Barrat, "Plastic deformation mechanisms of semicrystalline and amorphous polymers," *ACS Macro Lett.*, vol. 4, no. 2, pp. 147–150, 2015.

[13] H. Tian, J. Yan, A. V. Rajulu, A. Xiang, and X. Luo, "Fabrication and properties of polyvinyl alcohol/starch blend films: Effect of composition and humidity," *Int. J. Biol. Macromol.*, vol. 96, pp. 518–523, 2017.

[14] S. Santacruz, J. Ruales, and A. C. Eliasson, "Three under-utilised sources of starch from the Andean region in Ecuador: Part II. Rheological characterisation," *Carbohydr. Polym.*, vol. 51, no. 1, pp. 85–92, 2003.

[15] K. Thitipraphunkul, D. Uttapap, K. Piyachomkwan, and Y. Takeda, "A comparative study of edible canna (Canna edulis) starch from different cultivars. Part II. Molecular structure of amylose and amylopectin," *Carbohydr. Polym.*, vol. 54, no. 4, pp. 489–498, 2003.

[16] G.-X. Zou, P.-Q. Jin, and L.-Z. Xin, "Extruded Starch/PVA Composites: Water Resistance, Thermal Properties, and Morphology," *J. Elastomers Plast.*, vol. 40, no. 4, pp. 303–316, 2008.

[17] A. N. Frone, C. A. Nicolae, R. A. Gabor, and D. M. Panaitescu, "Thermal properties of water-resistant starch - Polyvinyl alcohol films modified with cellulose nanofibers," *Polym. Degrad. Stab.*, vol. 121, pp. 385–397, 2015.

[18] J. E. Barrera, J. A. Rodríguez, J. E. Perilla, and Nè. A. Algecira, "Estudio de la degradación térmica de poli(alcohol vinílico) mediante termogravimetría y termogravimetría diferencial," *Rev. Ing. E Investig.*, vol. 27, no. 2, pp. 100–105, 2007.

[19] I. Gómez *et al.*, "Thermal degradation study of PVA derivative with pendant phenylthionecarbamate groups by DSC/TGA and GC/MS," *Polym. Degrad. Stab.*, vol. 112, pp. 132–136, 2015.

[20] G.-X. Zou, P.-Q. Jin, and L.-Z. Xin, "Extruded Starch/PVA Composites: Water Resistance, Thermal Properties, and Morphology," *J. Elastomers Plast.*, vol. 40, no. 4, pp. 303–316, 2008.

[21] M. C. Popescu, B. I. Dogaru, M. Goanta, and D. Timpu, "Structural and morphological evaluation of CNC reinforced PVA/Starch biodegradable films," *Int. J. Biol. Macromol.*, vol. 116, pp. 385–393, 2018.

[22] H. P. Nguyen Vu and N. Lumdubwong, "Starch behaviors and mechanical properties of starch blend films with different plasticizers," *Carbohydr. Polym.*, vol. 154, pp. 112–120, 2016.

Evaluation of the Synergistic Effect of *Daucus carota* Honey and Essential Oils against *Candida albicans*

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ABSTRACT

The resistance of pathogenic fungal strains to the commonly used antifungal has necessitated a search for novel types of antifungal agents. The main objective of this study was to investigate the antifungal activities of essential oils (EOs) of medicinal plants (*Mentha pelugium Eugenia caryophyllata, Pelargonium graveolens*) and wild carrot *(Daucus carota*) honey when used jointly by the determination of MIC (Minimum Inhibitory Concentration) against *Candida albicans*. The result of our study indicated that the essential oils and honey are efficient against the tested strain. Honey MIC value was 6% (vol/vol), whereas the MIC values of EOs were 0.4µl/ml for *Pelargonium graveolens* and *Mentha peligium* EOs (vol/vol) and 0.33 µl/ml for *Eugenia caryophylatta* EO. When honey and EOs are used jointly, we noticed a decrease of the MIC values which is may be due to their synergistic effect. These preliminary results suggest that honey and EOs could be used together to manage superficial fungal infections.

I. Introduction:

Candida species are the most common pathogens fungal responsible for the majority of human infections ranging from localized superficial to systemic candidiasis [1]. This species are considered important pathogens due to their versatility and ability to survive in various anatomical sites. *Candida albicans* is the predominant cause of invasive fungal infections and represents a serious public health challenge with increasing medical and economic importance due to the high mortality rates and increased costs of care and duration of hospitalization. In recent years there is an increase incidence of drug resistant pathogens and the toxicity of existing antifungal compounds [2].Hence, there is a great demand for novel antifungal agents, justifying the intense search for new drugs that are more effective and less toxic than those already in use [3].This situation has drawn attention towards the antimicrobial activity of natural products [2].This products have been used for thousands of years in folk medicine for several purposes. They are both fundamental sources of new chemical diversity and integral components of today's pharmaceutical compendium [4]. Natural products, either as pure compounds such as honey or as standardised plant extracts, provide useful opportunities for new drug leads because of the matched less availability of chemical diversity. Honey is a natural product that has been used for its antifungal activity and its antimicrobial properties have been extensively reviewed [5]. The essential oils from many plants are known to possess antibacterial and antifungal activities [6].The aim of this study was to evaluate the synergistic action between honey and essential oils against *Candida albicans* and to investigate their joint potential use as an alternative for the treatment of infectious diseases.

II. Materials and methods

A. Honey sample

Monofloral honey sample (wild carrot honey: *Daucus carota* L) was directly provided by a local beekeeper from Chlef in western Algeria during the year 2014.

B. Physicochemical analysis of honey sample

Moisture in honey was determined in a refractometer (Jena 181282, Carl Zeiss, Oberkochen, Germany), and the pH of the honey solution was measured by a pH meter (CG840 Schott, Gerate GmbH, Hamburg, Germany). HMF content was measured according to the method of White [7] and was based on the determination of UV absorbance of HMF at 284 nm. The results are expressed in milligrams per kilogram (mg/Kg).

- *HPLC Analysis for sugars of honey sample*

Sugars and organic acids were analyzed by a Hewlet-Packard (1090) liquid chromatograph equipped with a photodiode array detector (PDA) and a Waters 410 differential refractometer (Milipore Corp., Milford, MA) connected in series. Data were processed using a Hewlet-Packard 85-B computing system and a Beckman Analogue Interface Module 406 with Gold V.711 software. Isocratic separation of the compounds was carried out at a flow rate of 0.4 mL/min on a stainless steel Ion-300 column (300 mm ¥ 7.8 mm, 10 mm) containing a cation-exchange polymer in the ionic hydrogen form, combined with an IonGuard GC801 precolumn (Interaction, San Jose, CA). Filtered (0.22 mm nylon) and degassed 0.0085 mol/L H2SO4 solution was used as the mobile phase. Both columns were maintained at 23C. Samples were dissolved in mobile phase, filtered through a micro-filter (politetrafilvoretileno [PTFE] or Teflon, 4 mm, 0.22 mm) and 20 mL (50% of total sample volume before filtration) was injected. The post column effluent was introduced in sequence into the PDA detector (scanning range 210–300 nm; 1.2 nm resolution) and a refractive index detector (sensitivity setting 16x, [8].

C. Essential oils extraction

The aerial plant parts (leaves and flowers, 30 g) of *Mentha pelugium* and the flower buds of clove (*Eugenia caryophyllata*) were dried at room temperature, hydro distilled for 3 h using a Clevenger type apparatus (British Pharmacopoeia, 1998). The EOs were dried over anhydrous sodium sulfate and stored in the dark at 2- 4°C. The yield of the essential oils was 1.56% and 7.45% (v/w) for *M. pelugium* and *E. caryophyllata* respectively. Except *Geranium (Pelargonium graveolens)* EO which was purchased from Algeria, the plants were obtained from a local store during the year 2014.

D. Gas chromatography-mass spectrometry (GC-MS):

GC analysis was carried out using a Shimadzu 2010 Plus gas chromatograph coupled to a Shimadzu QP2010 Ultra mass selective detector. The separation was performed by means of a Restek Rxi-5MS capillary column, 60 m lenght, 0.25 mm i.d. and a 0.25 µm phase thickness. The split mode was used. The oven program was as follows: Initial temperature was 60 ^{o}C for 2 min, which was increased to 240 ^{o}C at 3 ^{o}C min^{-1}, 250 ^{o}C was maintained for 4 min. Helium (99.999%) was used as carrier gas with a constant flow-rate of 1 mL min^{-1}. Detection was carried out in electronic impact mode (EI); ionization voltage was fixed to 70 eV. Scan mode (40-450 *m/z*) was used for mass acquisition. The volatile compounds were identified by comparison of their retention indices (relative to C7-C30 alkane standards), and matching mass spectral data with those held in FFNSC1.2 and W9N11 library of mass spectra and literature comparison [9].

E. Evaluation of the antifungal activity:

- *Fungal strain and inoculum standardization*

Candida albicans was kindly provided by a medical analyses laboratory it was isolated from a vaginal sample. Prior to the experiment the strain was maintained by subculture in the specific media; the inoculum suspension was obtained by taking five colonies from 48hours cultures. The colonies were suspended in 5 ml of sterile saline (0.85% NaCl) and shacked for 15 seconds. The density was adjusted to the turbidity of a 0.5 McFarland Standard (equivalent to 1×10^8 cfu/mL).

- *Minimum Inhibitory Concentration Measurement (MIC)*

The MIC of honey and essential oils have been determined, separately, using an agar incorporation technique method. Honey was added in increasing quantities (v/v) into media for a final volume of 5 ml. Essential oils were incorporated into Mueller-Hinton media. The mixture was shacked moderately and poured into plates, then standard inoculum of 0.5 McFarland of fungal strain was inoculated and the plates were incubated at 32°C for 48 hours. The MIC was determined based on the lowest concentration of honey and essential oils that inhibited the growth of tested organism.

F. Minimum Synergistic Inhibitory Concentration Measurement (MSIC)

To determine the minimum synergistic inhibitory concentration, volumes of honey were mixed with volumes of essential oils lower than the MIC values determined in the first step and then incorporated into Mueller-Hinton media. The mixture was shacked moderately and poured into plates, then standard inoculums of 0.5 McFarland of the fugal strain were inoculated and the plates were incubated at 32°C for 48 hours.

j. Statistical analysis

Isobolographic analysis was carried out using Statistica® 7software to measure the synergistic antifungal action of honey and EOs against the tested fungal.

III. Result and discussion:

Table 1 summarizes the physicochemical values of *Daucus carota* honey

TABLE 1. Values of physicochemical properties of *Daucus carota* honey

Honey	pH	Water content	HMF (mg/Kg)	Glucose (mg/g)	Maltose (mg/g)	Saccharose (mg/g)	Fructose (mg/g)
Daucus carota	4.62	16.4	13.98	326.26	37.16	11.67	406,49

The pH value of our sample *Daucus carota* honey was 4.62 which confirmed that this variety was acidic in nature. The acidity of the honey may be due to the presence of organic acids such as gluconic acid and also due to phosphate and chloride ions [10]. The pH acid of our honey was consistent with the results reported by various studies Kamboj et al [11]; Nayik and Nanda [12]; Shobham et al [13].This low pH inhibits the growth of microorganisms and influences the texture and stability of the honey sample [14].

Water is the second largest constituent of honey. The moisture content is one of the most important characteristics influencing physical properties of honey such as viscosity and crystallization, as well as other parameters: color, flavor, taste, specific gravity, solubility and conservation. Moisture content of honey is a limiting factor in determination of its quality, stability and spoilage resistance against yeast

fermentation. The higher moisture content is the higher probability of honey fermentation during storage [15].

The moisture content of our honey sample was below 20% maximum value allowed by Codex standards (Table 01). Such results were also observed by Omafuvbe and Akanbi [16] Nayik and Nanda [12]; Shobham et al [13].

Tosi et al [17] reported that hydroxymethylfurfural (HMF) as a quality parameter to check the honey freshness and high temperature processing. The higher value of HMF indicates overheating during processing, prolonged storage or adulteration with invert sugar. Our honey sample showed an HMF level lower than the limit (40 mg/kg), recommended by the Codex Alimentarius [18]

The glucose content in our honey was lower than the fructose content which indicated the natural feeding of honey colonies and confirmed the high quality of studied type of honey. These obtained results supported the previous several studies on different honey types (Buba et al., 2013[19]; EL-Metwally, 2015[20]; El Sohaimy et al 2015[15]).

Saccharose content is important to detect heavy sugar feeding of the bees or adulteration by direct addition of saccharose. According to some studies, the amount of sucrose has been used to discriminate the adulteration of honey samples by sugar syrups. For example, supplementary feeding of honey bees with sucrose syrup caused a higher sucrose level in honey. It comprises a little over 1% of the composition of honey [21].This seems to be the case of our honey.

The main compounds identified in the tested essential oils are showed in Table 02

TABLE 2. Chemical composition of essential oils (RI-retention index)

Sample Code	Name of Compound	Area (%)	RI
Eugenia caryophyllata			
1	*α* –Pinene	0.27	944
2	Benzaldehyde	0.97	971
3	Trimethylbenzene	0.45	1003
4	Eucalyptol	0.42	1041
5	Benzenepropanol	0.88	1170
6	Endo-Borneol	0.13	1175
7	Benzylidene malonaldehyde	0.37	1229
8	Benzylidene acetaldehyde	57.75	1279
9	Bornyl acetate	0.25	1294
10	Eugenol	2.34	1365
11	*E*-caryophyllene	0.17	1434
12	Alloaromadendrene	7.99	1762
13	*β* –Cedrene	0.63	1772

14	Pentacosane	4.03	2506
15	10, 12-Tricosadiynoic acid methyl ester	23.33	2609
Mentha pulegium			
1	p-Menthan-3-one	5.32	1163
2	p-Menth-4(8)-en-3-one	23.81	1249
3	Cinnamaldehyde	60.39	1278
4	p-Beritone	7.16	1352
5	Pentocosene	3.32	2500
Pelargonium graveolens			
1	Benzene 1,3,5 trimethyl	13.86	1002
2	Menthone	19.00	1161
3	Isomenthone	20.20	1172
4	Citronellol	30.81	1229
5	Citronellyl formate	16.13	1276

In our study we found that the major compounds of *Eugenia caryophyllata* essential oil are Benzylidene acetaldehyde 57.75%, 10, 12-Tricosadiynoic acid methyl ester 23.33%, Alloaromadendrene 7.99%, Pentacosane 4.03%, and Eugenol 2.34%. The chemical composition of our essential oil is different from result of other studies which showed that the major component of clove essential oil is usually eugenol, β- caryophyllene, α-humulene, caryophyllene oxide and eugenylacetate respectively, although different in concentration [22, 23-24]. The chemical composition of essential oils depends on climatic, seasonal and geographic conditions, harvest period and distillation technique [25].

The result of GC-Ms analysis showed that the major compounds of *Mentha pulegium* essential oil are cinnamaldehyde 60.39%, *p*-Menth-4(8)-en-3-on 23.81%, and p-Beritone 7.16%. In a study done by Nickavar and Fatemeh [26] they showed that 18 constituents were identified in the oil of *M. pulegium* and pulegone (48.7 %) and menthone (26.8 %) were found to be the main constituents. Other study done by Ainane et al [27] indicated that piperitone (31.27%) and piperitenone (22.98%) are the major compounds of the essential oil of *Mentha pulegium* grown in the region of Settat Morocco.

Citronellol, isomenthone, menthone and citronellyl formate are the most chemical compounds of our geranium essential oil. The main results of a study done by Mnif et al [28] showed that *P. graveolens* essential oil was characterized by the predominance of two compounds: citronellol and geraniol with respective amounts of 27.53 and 25.85 %.

Bigoset al [29] found in their study that citronellol 26.7% and geraniol 13.4% representing the major compounds of essential oil of geranium.

- *Antifungal effect of Daucus carota honey and essential oils*

Honey and all the essential oils were effective against the tested strain (*Candida albicans*) and the MIC values varied widely depending of the natural products. The MIC value of honey was 6%.The MIC values of EOs varied widely depending on the botanical origin. The EO from *E. caryophylata* was the most effective one with a MIC value of 0.3µl/ml as shown in the Table 03.

TABLE 3. MIC values of *Daucus carota* honey and essential oils against *Candida albicans*

	MIC values			
	Honey %	**Essential oils µl/ml**		
Substances	*Daucus carota* honey	*Geranium*	*Eugenia caryophylatta*	*Mentha pelugium*
Candida albicans	6%	0.4	0.33	0.4

The antifungal activity of honey is thought to be attributed to the high concentration of sugars and low content of water [30]. Several factors may influence this activity. These factors include its physico-chemical properties, botanical and entomological origin. In addition, there are a great variety of components, including phenolic acids, flavonoids and other biomolecules, in different honeys. Biological activities of honey is mainly attributed to the phenolic compounds [31]. The antimicrobial action of these compounds has been related to their ability to denature proteins, being generally classified as surface active agents [32]. The antifungal activity of honey against *Candida albicans* has been reported in many studies.

Estevinho et al [31] found in their study that lavender honey inhibited the growth of pathogenic yeasts *Candida albicans, Candida krusei, and Cryptococcus neoformans.*

In a study done by Anyanwu [32] he was evaluated the antifungal activity of honey samples against *Aspergillus niger, Aspergillus flavus, Penicillium chrysogenum, Microsporum gypseum, Candida albicans*, and *Saccharomyces* sp. The obtained results revealed that the honey samples showed varying levels of inhibitory activity at various concentrations against the tested fungi *M. gypseum* was the most sensitive of all the studied fungal, while *C. albicans* was the least sensitive.

Other study done by Al Zahrani et al [33] they evaluated the antimicrobial potency of four varieties of honey from different botanical and geographical origins (Manuka, Acacia, Lavender, Wild carrot) they found that all honey samples were effective against *Candida albicans.*

The result of a study done in Australia by Irish et al [34] indicated that four varieties of honey have an antifungal effect against *Candida* species (*C. albicans*, *C. glabrata* and *C. dubliniensis)*.

In a study done Koc et al [35] they evaluated the ability of honey samples from different floral sources to inhibit the growth of four yeast strains (*Candida albicans, C. krusei, C. glabrata* and *Trichosoporon* spp.). The result of this study indicated that all of the tested yeast strains were inhibited by honeys.

The result of our study (Table 03) indicated that all the tested essential oils were active against *Candida albicans*, this activity is attributed to the presence of small terpenoid and phenolic compounds [36]. It is generally assumed that the mechanisms by which the constituents of essential oils inhibit the growth of micro-organisms may be partially dependent on their hydrophobicity. It enables them to embed in the cell wall, damage the lipid layer of the cell membrane and mitochondria, impair enzyme systems and exhibit side effects on various proteins . Some of them inhibit microbial growth by causing also a global arrest in protein synthesis or inducing cytoplasm coagulation [06]. Various studies showed that essential oils have antifungal activity against *Candida albicans* .

Abdulaziz et al [1] found that both essential oils of *Rosemarium officinalis* and *Thymus vulgaris* are active against the selected fluconazole resistant *C. albicans* isolates.

Hammer et al [37] have investigated the antimicrobial activity of a large number of essential oils against a diverse range of organisms comprising Gram positive and Gram negative bacteria and yeast, they found that the essential oils of *Eugenia caryophyllata (clove oil), Pelargonium graveolens(Geranium oil) and Mentha piperita* have an antifungal effect against *Candida albicans.*

Budzyńska et al [38] found that essential oils of *Eugenia caryophyllata, Pelargonium graveolens and Mentha piperita* have an antifungal effect against *Candida* Spp (*C. albicans* strains (ATCC 10231, ATCC 90028) and 50 clinical isolates: *C. albicans* (n = 20), *C. glabrata* (n = 13), *C. kru sei* (n = 6), *C. parapsilosi*s (n = 5), C. *tropicalis* (n = 6)).

The result of a study done by Bhat et al [39] reveled that the essential oil of *Eugenia caryophylatta* had antifungal activity against the oral isolates of candida species (*Candida albican, Candida glabrata, Candida tropicalis*)

Ahmad et al [40] found in their study that Eugenol and methyleugenol the major compounds in the essential oils of many aromatic plants, such as clove (*Eugenia caryophyllata*) have antifungal effect against 64 fluconazole sensitive and 34 fluconazole resistant clinical Candida.

In other study done by Pinto et al [3] they found that essential oil of *Eugenia caryophyllata* and their major compounds eugenol showed a broad spectrum of activity against a variety of pathogenic yeasts (*Candida albicans, Candida Krusei, Candida tropicalis Candida glabrata, Candida parapsilosis*) and filamentous fungi (*Epidermophyton floccosum ,Trichophyton rubrum,Trichophyton mentagrophytes, Microsporum canis, Microsporum gypseum, A. flavus , A. fumigatus, A. niger*) including fungi with decreased susceptibility to fluconazole.

In a study done by Matsuzaki et al [41] they confirmed the antifungal activities against *C. albicans* of seven essential oils from aromatic plants, lemongrass (*Cymbopogon citrates*), eucalyptus (*Eucalyptus globules*), tea tree (*Melaleuca alternifolia*), peppermint (*Mentha piperita*), sweet marjoram (*Origanum majorana*), geranium (*Pelagonium graveolens*) and rosemary (*Rosmarinus officinalis*).

Adding EOs to honey resulted in a significant decrease in the MIC of EOs and honey.

Isobolographic representations show a synergistic action between honey and the different varieties of EOs in term of antifungal activity (Figures 1, 2, 3).

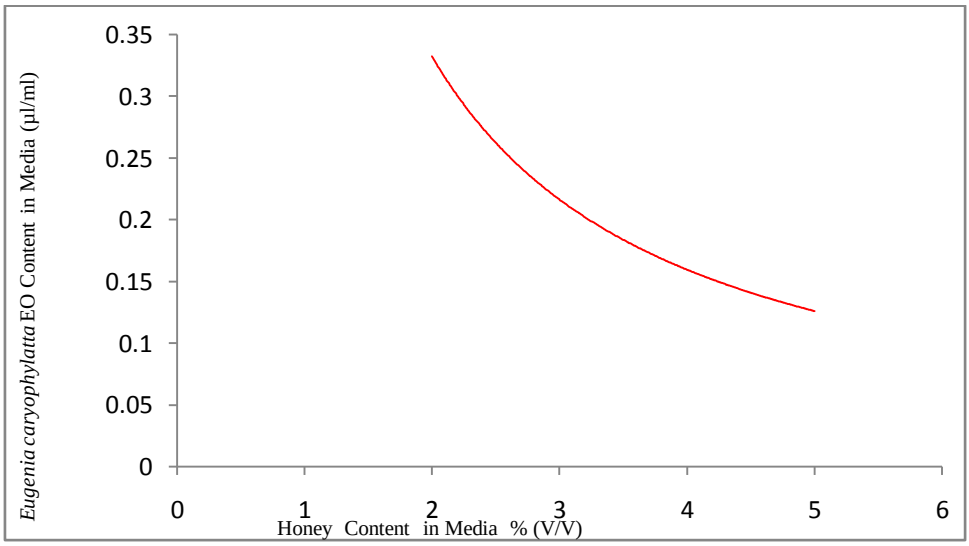

Fig 1: Isobologram representing synergistic effect of *Eugenia caryophylatta* essential oil and *Daucus carota* honey against *Candida albicans.*

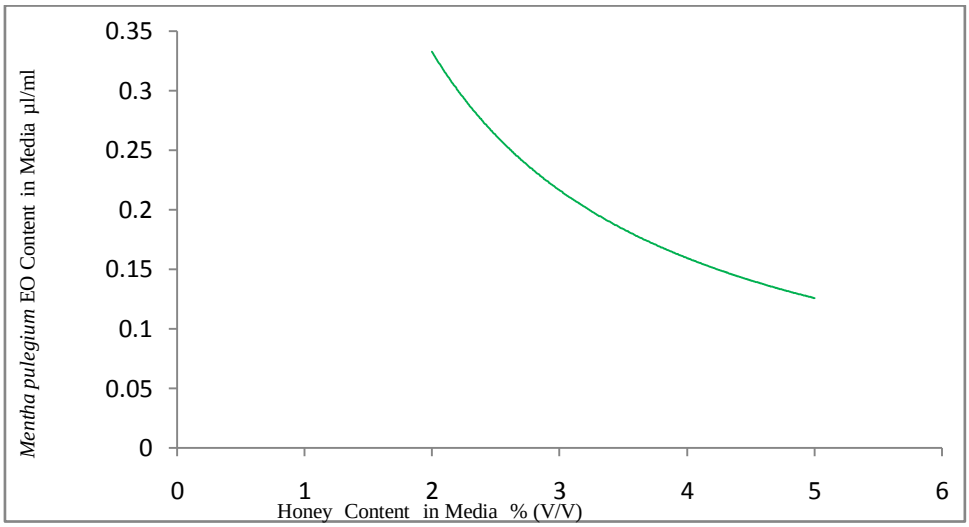

Fig 2: Isobologram representing synergistic effect of *Mentha pulegium* essential oil and *Daucus carota* honey against *Candida abicans.*

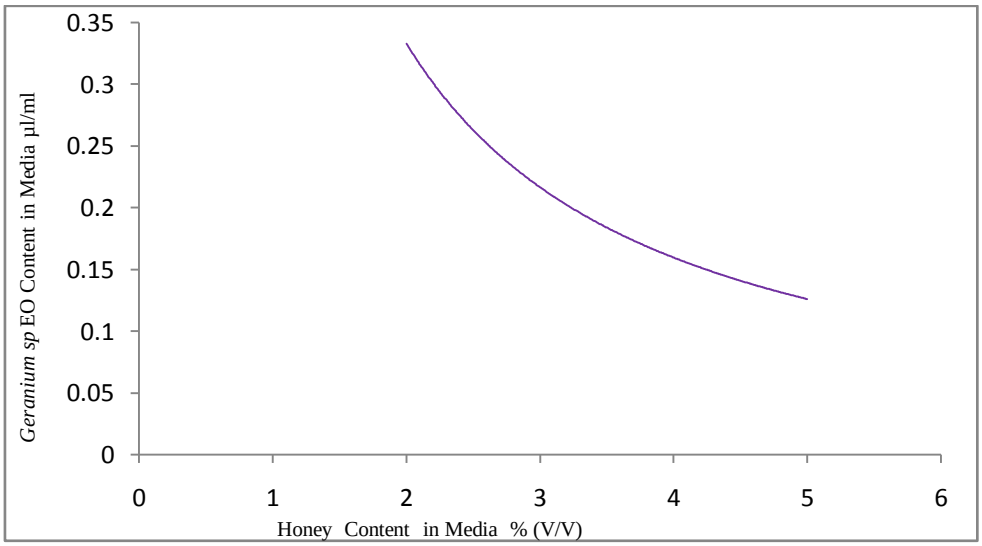

Fig 3: Isobologram representing synergistic effect of *Geranium sp* essential oil and *Daucus carota* honey against *Candida albicans*

The synergistic effect of honey and other natural compounds against bacteria and fungi has been reported by many studies .Azahrani et al [5] found in their research that *Daucus carota* honey and five essential oils types (*Thymus fontanesii*, *Thymus vulgaris,Origanum vulgaris, Eugenia caryophyllata* and *Geraniuma*) have a synergistic antifungal effect against two fungi strains *Aspergillus niger* and *Aspergillus flavus.*

The result of a study done by Boukraa et al [42] indicate that five varieties of honey from different botanical origins :Manuka honey (*Leptospermum scoparium*) Acacia honey (*Acacia*),wild carrot honey (*Daucuscarota* L),Berringa honey (*Leptospermum polygalifolium*) Sidr honey (*Ziziphus zizyphus*) and essential oils extract from four medicinal plants *Thymus vulgaris,Thymus fontanesii Origanum vulgare* and *Eugenia caryophyllata* have a synergetic antibacterial effect against *Pseudomonas aeruginosa* ATCC 27853.

The result of a research done by Khosravi-Darani et al [43] showed that three kinds of honey of Iran and alcoholic extract of mint and zataria, as well as extract and starch of ginger have a synergistic antimicrobial action against *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli* and *Candida albicans.*

The result of a study done by Abdellah et al [44] showed that *Daucus carota* honey and the powder of *Thymus ciliatus* acted synergistically against three pathogenic bacteria, namely *Staphylococcus aureus* OxaR ATCC 43300, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853.

In a study done by Boukraa et al [45] they found that honey and starch have a synergistic action against *Candida albicans.*

Another study done by Boukraa et al [46] they demonstrated that honey and starch have a synergistic action against *Pseudomonas aeruginosa.*

Boukraa and Amara [47] found that three varieties of honey and starch have a synergistic effect against *Staphylococcus aureus* and *Escherichia coli.*

Boukraa [48] indicates that four varieties of honey from different botanical origins and royal jelly have a synergistic antibacterial effect against *Pseudomonas aeruginosa* ATCC 27853.

IV. Conclusion

The extensive use of antifungal chemicals in medical area has led to the selection of resistant fungal strains. So, to overcome this problem, it is necessary to find out alternative medicines that could be efficient and safe for use. The re-emergence of using natural antifungal compounds is not new. Honey and EOs are natural products which may be used jointly to boost their antifungal action against pathogenic fungi.

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References

[1] S M. Abdulaziz, I A. Shaswary. A M. Aso, In vitro antifungal activity of essential oils from local plants against fluconazole-resistant oral *Candida albicans* isolates. Zanco J Med Sci, Vol. 19, pp. 965-971, July 2015.

[2] JC O. Sardi , L. Scorzoni, T. Bernardi, A M. Fusco-Almeida, MJS. Mendes Giannini, Candida species: current epidemiology, pathogenicity, biofilm formation, natural antifungal products and new therapeutic options. *J* Med *Microbiol*,Vol. 62, pp.10-24, January 2013.

[3] E. Pinto, L. Vale-Silva, C. Cavaleiro, L.Salgueiro, Antifungal activity of the clove essential oil from *Syzygium aromaticum* on *Candida, Aspergillus* and dermatophyte species. *J* Med Microbiol. Vol.58, pp. 1454–1462, November 2009.

[4] H. Mehrgana, F. Mojab, S. Pakdaman and M. Poursaeed, Antibacterial activity of *thymus pubescens* methanolic extract. Iran J Pharm Res,Vol. 7, pp.291-295, May 2008.

[5] HA. Alzahrani, L. Boukraa, F. Abdellah, B.Bakhotmah, S M. Hammoudi, *In vitro* Synergistic Action of Honey and Essential Oils against Two Species of *Aspergillus:* A Preliminary Study. Br J Pharm Res ,Vol. 4, pp. 93-100, October 2014.

[6] F.Bakkali, Biological effects of essential oils-*a* review. Food Chem Toxicol, Vol.46, pp. 446-475, February 2008.

[7] JW. White, Methods for determinging carbohydrates, hydroxymethylfurfural, and proline in honey: Collaborative study. J Assoc Off Anal Chem, Vol. 62,pp. 515–526. May 1979.

[8] AG. Pérez, JJ. Rios, C. Sanz, JM. Oléas, Rapid determination of sugars, nonvolatile acids, and ascorbic acid in strawberry and other fruits. J Agric Food Chem,Vol.45, pp. 3545–3549. February 1997.

[9] C. Bicchi, E. Liberto, M. Matteodo, B. Sgorbini, L. Mondello, BA. Zellner, R. Costa, P. Rubiolo, Quantitative analysis of essential oils: a complex task. Flavour Fragr J,Vol. 23,pp. 382-391.October 2008.

[10] V. Nanda, BC. Sarkar, HK. Sharma, AS. Bawa, Physico-chemical properties and estimation of mineral content in honey produced from different plants in Northern India. J. Food Comp. Anal, Vol.16, pp. 613-619. October 2003.

[11] R. Kamboj, BM. Bera, V. Nanda, Evaluation of physico-chemical properties, trace metal content and antioxidant activity of Indian honeys. Inter J Food Sci Technol, Vol. 48, pp.578–587. November 2013.

[12] G A. Nayik, V. Nanda, physico-chemical, enzymatic, mineral and colour characterization of three different varieties of honeys from kashmir valley of india with a multivariate approach. Pol J Food Nutr Sci, Vol. 65, pp. 101–108. February 2015.

[13] Shobham , K K.Chitluri, J.Nayar, Physico-Chemical Analysis of Some Commercial Honey Samples from Telangana. Indian J Nutri, Vol. 4 pp.153. February 2017.

[14] V. Baroni, C. Arrua, ML. Nores, P. Faye, MDP. Diaz, Composition of honey from Córdoba (Argentina): Assessment of North/South provenance by chemometrics. Food Chem, Vol. 114, pp. 727-733. May 2009.

[15] SA. El Sohaimy, SHD. Masry , MG. Shehata , Physicochemical characteristics of honey from different origins. Ann Agric Sci, Vol. 60, December 2015.

[16] BO. Omafuvbe , OO. Akanbi , Microbiological and physico-chemical properties of some commercial Nigerian honey. Afr J Microbiol Res ,Vol.3,pp. 891- 896. December 2009.

[17] E. Tosi, Ciappini M., E. Re, H. Lucero, Honey thermal treatment effects on hydroxymethylfurfural content. Food Chem, Vol.77, pp.71–74.August 2002.

[18] FAO and WHO. CODEX STAN 12-1981: Codex Standard for Honey. Codex Alimentarius Commission. 2001.

[19] F. Buba, A.Gidado, A. Shugaba, Analysis of biochemical composition of honey samples from North-East Nigeria. Biochem. Anal. Biochem. Vol.2 ,p 139. September 2013.

[20] AAE. EL-Metwally, Factors Affecting the Physical and Chemical Characteristics of Egyptian Beehoney. Ph. D. Thesis, Fac. Agric. Cairo Univ., 320p. 2015.

[21] M. Ozcan, D. Arslan, AC. Durmus,. Effect of inverted saccharose on some properties of honey. Food Chem.Vol. 99,pp. 24–29. 2006.

[22] HK. Alma, M. Ertas, S. Nitz, H. Kollmannsberger, Chemical composition and content of essential oil from the bud of cultivated Turkish clove (Syzigium aromaticum L.), BioResources, Vol. 2, pp.265-269. 2007

[23] Md N I. Bhuiyan, J. Begum, N Ch. Nandi, F. Akter, Constituents of the essential oil from leaves and buds of clove (*Syzigium aromaticum*, L. Alston). Afr J Plant Sci, Vol. 4, pp. 451-454. 2010.

[24] G. Razafimamonjison, M. Jahiel, TH. Duclos, P. Ramanoelia, F. Fawbush, P. Danthu, Bud, leaf and stem essential oil composition of *Syzigium aromaticum* from Madagascar, Indonesia and Zanzibar, Int J Basic Appl Sci, , Vol. 3,pp. 224-233. 2014.

[25] H. Karataş, and S.Ertekin, Antimicrobial activities of the essential oils of four *Salvia* species from Turkey. J. Med *Plants* Res, Vol.4,pp. 1238-1240. June 2010.

[26] R.Nichavar, F.Jabbareh, Analysis of the essential oil from *Mentha pulegium* and identification of its antioxidant Constituents.Journal of essential oil research,Vol.21, pp.223-229. April 2018.

[27] A. Ainane, F. khammour ,M. Elkouali, M.Talbi, E. Abba, S. Cherroud, A. Elyaacoubi, K. Oumaskour, N.Boujaber , S. Benhar , T.Ainane, Chemical Study by GC-MS of the Essential Oils of Certain Mints Grown In the Region of Settat (Morocco): *Mentha Piperita, Mentha Pulegium* and *Mentha Spicata*. Drug Des Int Prop Int J,Vol. 1, pp.124-127. June 2018.

[28] W. Mnif, W. Dhifi , N. Jelali ,H. Baaziz , A. Hadded , N. Hamdi, Characterization of Leaves Essential oil of Pelargonium graveolens Originating from Tunisia: Chemical Composition, Antioxidant and Biological Activities. Journal of essential oil-bearing plants JEOP, Vol. 14, pp.761-769. March 2013.

[29] M. Bigos, W. Małgorzata, K. Danuta, M .Sienkiewicz, Antimicrobial activity of geranium oil against clinical strains of *Staphylococcus aureus*. Molecules, Vol.17, pp. 10276-10291. September 2012.

[30] P. Molan, The antimicrobial activity of honey. Bee world, Vol.73,pp 5-28. 1992.

[31] M L. Estevinho, S E. Afonso, X.Feás, Antifungal effect of lavender honey against *Candida albicans, Candida krusei* and *Cryptococcus neoformans*. J.Food Sci.Technol,Vol.48, pp.640-643.February 2011.

[32] CU. Anyanwu, Investigation of *in vitro* antifungal activity of honey. J. Med. Plants Res.Vol.6, pp.3512-3516. May 2012.

[33] H.A, Alzahrani, R. Alsabehi, L. Boukraâ, F. Abdellah, Y. Bellik, B. Bakhotmah, Antibacterial and Antioxidant Potency of Floral Honeys from Different Botanical and Geographical Origins.Molecules Vol.17, pp.10540-10549. October 2012.

[34] J. Irish, D A. Carter , T.Shokohi, S E. Blair, Honey has an antifungal effect against *Candida* species. Med Mycol, Vol.44, pp. 289-291. May 2006.

[35] AN. Koc , S. Silici , BD. Ercal , F. Kasap , Oz HT. Hörmet, H.Mavus-Buldu, Antifungal activity of Turkish honey against Candida spp. and Trichosporon spp: an in vitro evaluation. Med Mycol, Vol. 47, pp. 707-712. 2009.

[36] MJ. Pelczar, ECS. Chan, NR. Krieg, Control of microorganisms, the control of microorganisms by physical agents. In Microbiology, pp.469–509. 1988.

[37] KA. Hammer, CF. Carson, TV. Riley, Antimicrobial activity of essential oils and other plant extracts. J. Appl. Microbiol, Vol.86, pp.985–990. February1999.

[38] A. Budzyńska, B. Sadowska, G. Lipowczan, A. Maciąg, D. Kalemba, B. Różalska, Activity of Selected Essential Oils against *Candida* spp. strains. Evaluation of New Aspects of their Specific Pharmacological Properties, with Special Reference to Lemon Balm., Adv Microbiol. 3, 317-325. July 2013.

[39] V. Bhat , S.M. sharma , V. shetty , CS. shastry , V Rao ,S M. Shenoy , S. Saha, S.Balaji, Screening of selected plant essential oils for theirantifungal activity against candida species isolated from denture stomatitis patients. Nitte university journal of health science. Vol.4,pp 2249-7110. March 2014.

[40] A. Ahmad, A. Khan, L. Ahmad Khan, N. Manzoor, In vitro synergy of eugenol and methyleugenol with fluconazole against clinical Candida isolates. J Med Microbiol, Vol.59, pp.1178–1184. October 2010.

[41] Y. Matsuzaki, T. Tsujisawa, T. Nishihara, M. Nakamura, Y. Kakinoki, Antifungal activity of chemotype essential oils from rosemary against *Candida albicans.* Open Journal of Stomatology,Vol. 3, pp. 176-182. 2013.

[42] L. Boukraâ, H. A, Alzahrani, F. Abdellah, B. Bakhotmah,SM, Hammoudi, Synergistic effect of Monofloral Honeys and Essential Oils against *Pseudomonas aeruginosa.* Br Microbiol Res J, Vol.3, pp. 564-573. 2013.

[43] K. Khosravi-Darani, R. Khaksar, S. Esmaeili, F. Seyed-Reihani, A. Zoghi, S. Shahbazizadeh, Antifungal and Anti-bacterial Synergistic Effects of Mixture of Honey and Herbal Extracts. Zahedan. J. Res. Med. Sci, Vol.15,pp. 30-33. August 2013.

[44] F. Abdellah, L. Boukraâ, S M. Hammoudi, H A. Alzahrani, B. Bakhotmah, Synergistic Effect of Honey and *Thymus Ciliatus* Against Pathogenic Bacteria. *Open Nutraceuticals* J, Vol.5, pp. 174-178. July 2012.

[45] L. Boukraa, H. Benbarek, A. Moussa .Synergistic action of starch and honey against *candida albicans* in correlation with diastase number. Braz J Microbiol,Vol.39, pp. 40-43. January 2008.

[46] L. Boukraâ, H. Benbarek, S. Aissat, Synergistic action of starch and honey against *Pseudomonas aeruginosa* in correlation with diastase number. J Altern Complement Med, Vol. 14, pp. 181-184. March 2008.

[47] L.Boukraâ, K. Amara, Synergistic Effect of starch on the antibacterial activity of honey. J Med Food, Vol.11,pp. 195-198.March 2008.

[48] L .Boukraâ, Additive activity of royal Jelly and honey against *Pseudomonas aeruginosa*. Altern Med Rev, Vol.13, pp. 330-333.2008.