Plant Archives Vol. 21, Supplement 1, 2021 pp. 586-591 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

Journal homepage: http://www.plantarchives.org
doi link : https://doi.org/10.51470/PLANTARCHIVES.2021.v21.S1.089

STUDY OF THE BIOLOGICAL ACTIVITY OF CASTOR OIL EXTRACT AND ITS SYNTHESIZED AMIDES ON BACTERIA

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ABSTRACT

The present paper included the use of some natural and environmentally friendly products extracted oil of castor seed and its amide. Chemical and physical characterization of the extracted oil and its amide were studied. Amide was prepared from the reaction of castor oil with ethylene di-amine. Infrared spectra used to diagnosis extracted oil of castor seed, while H.NMR and FTIR spectra were used to diagnosis synthesis amide from castor oil. The biological activity of the extracted oil and its amide were evaluated in different concentrations of 50,100 and 200 µg /ml on two types of bacteria (*Pseudomonas aeruginosa*, *Staphylococcus aureus*). The results show antibacterial activity and different inhibition zones. The diameter of the inhibition areas increases with increasing concentration.

***Keywords*:** castor oil seeds, extraction, amide synthesis, biological activity, medical plants.

Introduction

Medicinal plants represent a gift of nature to humans because they contribute to improving healthy and disease-free lives. Plants used as drugs for humans for thousands of years, also an important source of a varied range of secondary metabolites utilized in pharmaceuticals, agrochemicals, flavors, fragrances, dyes, biocides and food additives (Al-Snafi, 2017; Al-Snafi, 2017). Organization of world health estimates that 80% of the citizens in developing countries utilizes only traditional medicine for primary health care. More than half of the world's people still depends entirely on plants for its medicines, and plants provide the active components of more traditional medicines (Kumar and Navaratnam, 2013). Essential oils of plants including gram-negative and gram-positive bacteria, so several countries have preserved research programs to test traditional medicines for antimicrobial practice, such as in India, Africa, Palestine, Cuba, Honduras, Jordan, and Italy (Ambindei *et al.*, 2017). Oil of Castor Seed is a source of commercial hydrogenated fatty acids. The presence of ricinoleic acid in castor oil gives its unique properties and extraordinary work. It is different from other oils that have a high acetyl or hydroxyl value and form oil of comparable iodine value with a high viscosity and specific gravity (Jumat *et al.*, 2010). Unlike other oils, they can be mixed with alcohol, but only slightly soluble in the oil ether at room temperature. Castor oil also has good wet and lubrication characterizations, as well as a notable ability to color and disperse dyes, dyes and fillers (Wang *et al.*, 2013; Felipe *et al.*, 2013). So oil of castor seeds and its derivative (amide) were chosen to be tested for its antimicrobial properties due to its availability and potential as a medicinal plant. Generally, Castor oil can be used in small doses in clinical trials.

This study aims to test bioactivity of extracted oil from castor seeds and its synthesized amide.

Materials and Methods

Extraction process of castor oil

The extraction process of castor seeds was done by using Soxhlet apparatus. 200 ml of Petroleum ether was placed in around bottom then put 50gm of castor powder (grinder seeds by electric grinder). The extraction process lasts 6-10 hr. The solvent was removed by a rotary evaporator after ending extraction process (De Souza Schneider *et al.*, 2004; Athraa *et al.*, 2019). Extracted oil was diagnosed using the FTIR technique.

There are many Physical and Chemical characterizations of extracted castor oil were determined: Water Degumming (Patel *et al.*, 2016), specific gravity (Isah, 2006; Auta, 2013), pH value (Omari, 2015), determine the moisture in the castor seeds and the percentage of extracted oil (Omari *et al.*, 2015; Hameed *et al.*, 2003), acid value and determination of saponification value (Hindi and Dawoud, 2019; Kyari, 2008).

Amide preparation

A 24gm of Extracted oil of castor was reacted with 12gm of ethylene di ammine by using50 ml of xylene as a solvent in a four-neck round bottom flask fitted with electrical stirrer to solve the previous mixture. Extracted oil added as drops to the flask for one hour at approximately 110 °C with electrical stirring rate of 600 rpm. To convert the extracted oil into amides completely, the mixture was stirred electrically at 120 °C for five hours. Complete reaction was detected by TLC. Rotary evaporation used to remove the solvent. The synthesized amide was further purified in a chromatographic column of silica-gel using mixture of

trichlromethane ($CHCl_3$) and absolute methanol (CH_3OH) (V:V=6:1) with a yield of 95.3% the purified amide.

FTIR Spectrophotometric (Shimadzu FT-IR 8400S spectrophotometer) and Nuclear magnetic Resonance Spectroscopy (1H-NMR) spectra ((Bruker DRX System AL 500, 125 500, 125MHZ) were used to diagnosis synthesized amide.

Antibacterial Activity

Inhibitors consist of extracted oil of castor and its synthesized amides where tested to study the efficiency of these inhibitors on the bacteria through measuring the inhibition areas. Bacteria isolates gram- positive (*Staphylococcus aureus*) and gram-negative (*Pseudomonas aeruginosa*) were obtained from Hospital Hussein Education in Nasiriyah. Stock solution of 1% oil prepared through dissolving 100 mg of extracted castor oil in 10 ml of solution solvent (9 ml H_2O + 1 ml dimethyl sulphoxide (DMSO), DMSO solvent used to dissolve the extracted oil in water. Stock solution was diluted to 50, 100 and 200µg/ml. Three different tests concentrations were assessed for their antibacterial activity against strains of bacteria using disc diffusion method. Briefly, sterile 6 mm, filter paper disc was placed gently on MH agar freshly inoculated with bacteria, with the help of a sterile forceps to ensure complete contact with the agar surface. Extracted oil was applied onto each paper disc, followed by incubated at 37 °C for 24 hr. The antibacterial activity was evaluated by measuring the diameter of the inhibitory zone in term of millimeters and recorded (Tuchilus *et al.*, 2017; Al-Sehemi *et al.*, 2016).

Results and Discussion

In this section chemical, physical, and the bioactivity of extracted castor oil and its synthesized amide will be discussed.

Physical and chemical characterizations of extracted castor oil had been determined as shown in the following Table (1):

Table 1: Physical and chemical characterizations of extracted castor oil.

Material	A.V(mg/g)	S.V(KoH mg/gOil)	PH	Moisture %	Specific Gravity	Percentage extraction
Castor oil	0.42	178	6	1.9	0.95	(42-60)%

It was necessary to determine the moisture content of these oil seeds, because it shows the amount of water that must be removed before the process of extracting by soxhlet apparatus. Excess water in the seeds reduces the quality of castor oil produced by the action of microbes and lipase which found in castor seed. As shown in above table, the moisture percentage is within the global permission range (0.2-4.12)% (Salimon *et al.*, 2010).

Percentage of extraction as shown in Table (1) is butter than many studies like studies (James *et al.*, 1965; Akpan *et al.*, 2006). Saponification value represents an indicator of molecular weight or chain length of the fatty acids found in the oil. Saponification value of castor oil is 178 as shown in the Table (1). Various oils can be distinguished according to the specific gravity. The specific gravity of castor oil was found in the range 0.95-0.96.

pH value and an acid value of castor oil as shown in Table (1) are 6 and 0.42 mg / g. respectively. The acid value indicates the low level of hydrolysis because of lipase, strong phenolic anti-oxidants such as Butylated Hydroxyanisole (BHA) and Butylated Hydroxytoluene (BHT) in the oil. These values are small in comparison with the studies (Oliniyan, 2010; Muzenda *et al.*, 2012).

Diagnosis of castor oil composition

The composition of castor oil was diagnosed by the FTIR technique as shown in Figure (1). The results of FTIR spectra are shown in Table (2).

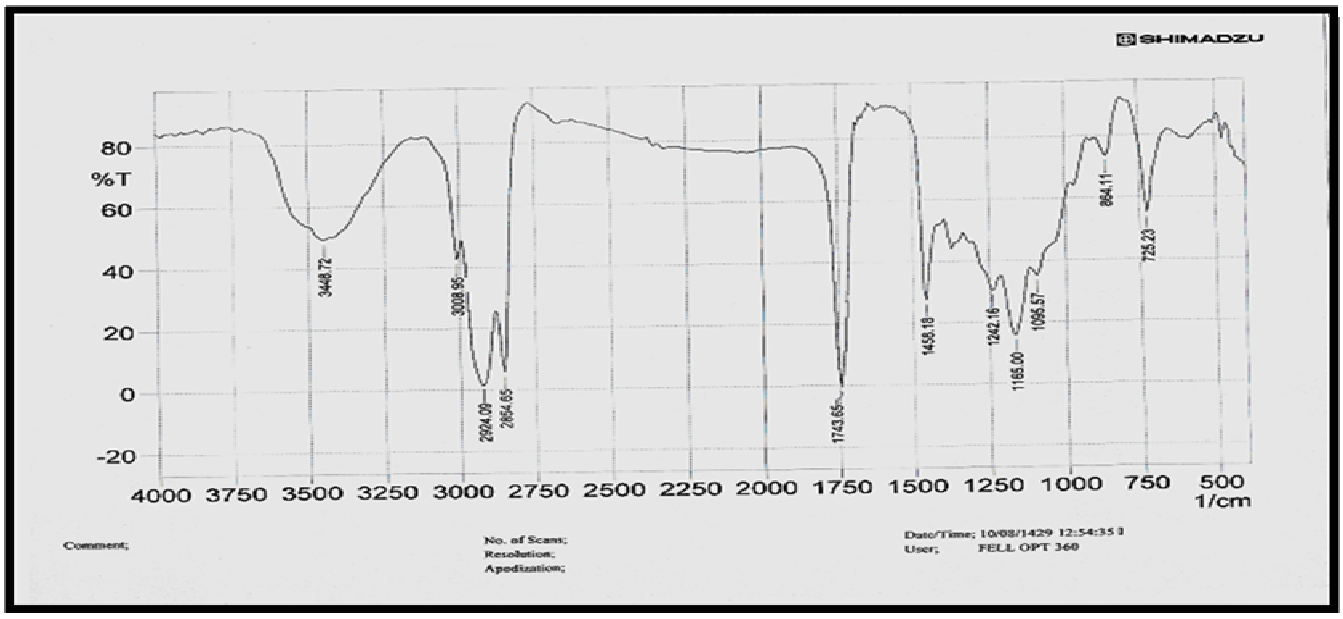


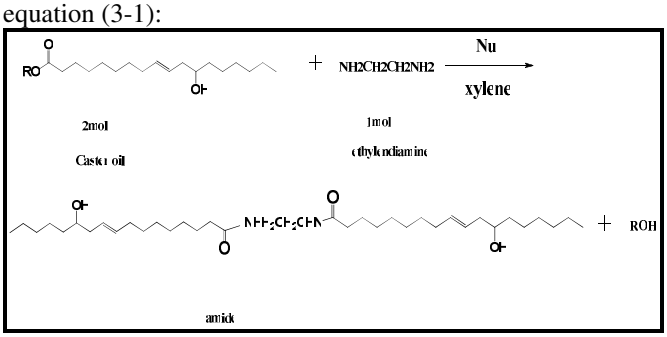
Fig. 1 : Infrared spectrum of castor oil

Table 2 : The results of FTIR spectra of castor oil

Functional groups	Castor oil
OH	3448.72 cm^{-1}
C=O	1743.65 cm^{-1}
CH2(Aliphatic)	2854-2924 cm^{-1}
CH2 bend	1458.18 cm^{-1}
CH3 bend	1380 cm^{-1}
C-O	1242.16 cm^{-1}
C-OH	1165 cm^{-1}

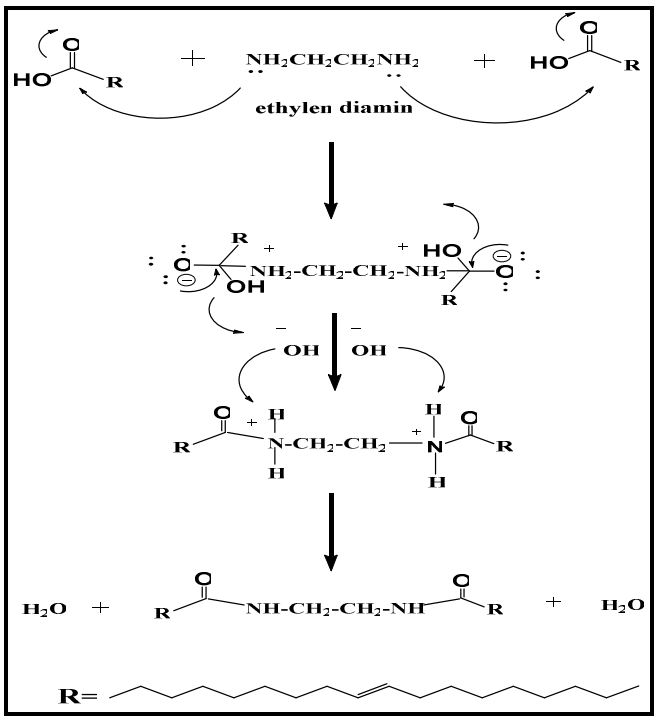
Synthesis of Amides

Amide was prepared by reacting of the extracted oil of castor seeds with ethylene diamine in the presence of xylene as a solvent according to the following reaction equation (3-1):



Equation (1): Preparation steps of amide.

Where the reaction takes place by the Nucleophilic attack of the nitrogen atom of the amine on the carbonyl carbon and the proposed mechanics are shown according to the following diagram (1):



Scheme (1): Mechanism preparation amide

Diagnosis of Amide by FTIR spectra and H1.NMR technique

The composition of synthesized from amide of castor oil was diagnosed by FTIR H1.NMR techniques as shown in Figure (2) and (3-3) respectively. The results of the diagnosis of FTIR spectra and H1.NMR of amide are shown in Table (2) and (3) respectively note that amid is symmetric.

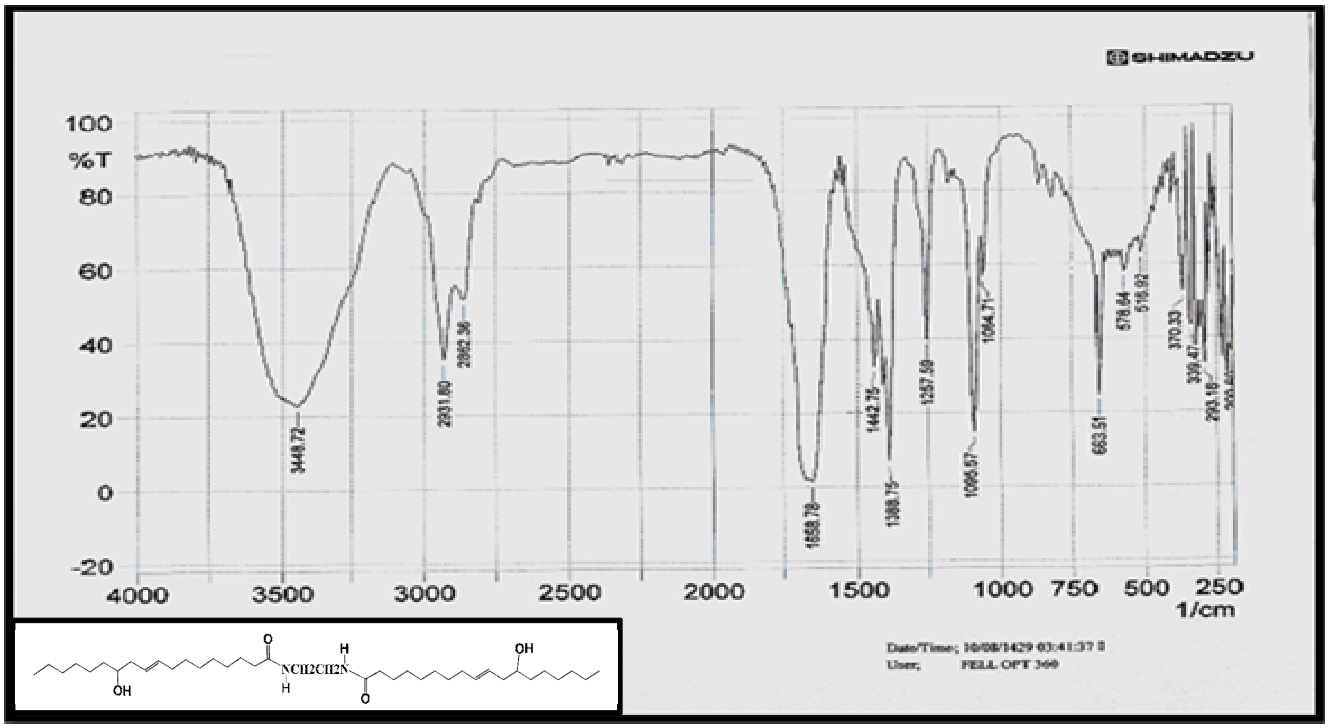


Fig. 2 : Infrared spectrum of Amide.

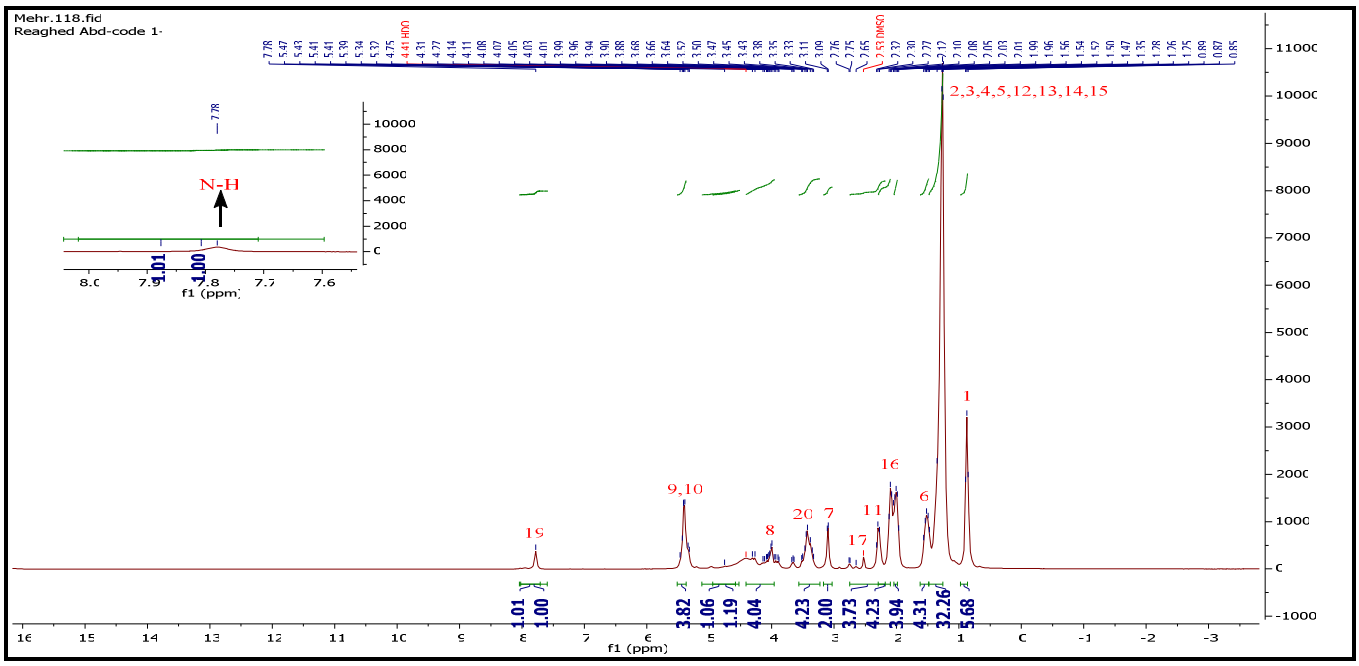
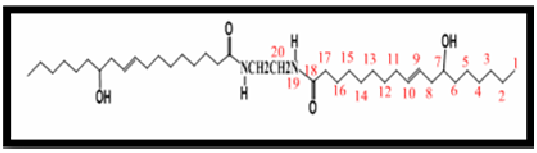


Fig. 3: H1.NMR of Amide

Table 3 : The results of diagnosis of amide by FTIR spectra

Functional group	Amide
N-H (amides)	3448.72 cm^{-1}
C=O (amide)	1658.78 cm^{-1}
C-H (alphatic)	2862.36-2931.80 cm^{-1}
C=C	1504.48
CH_2 bend	1442.75 cm^{-1}
CH_3 bend	1388.75 cm^{-1}
C-N	1095.57 cm^{-1}

Table 4 : The results of the diagnosis of amide by H1.NMR spectra

Amide	Nuclear magnetic resonance signals (ppm)
	7.65-7.96 (S,2H,NHamide),5.34-5.46(q,4H,CH=CH),4.61-5.03 (S,2H,OH),3.96-4.33(t,4H,CH_2),3.26-3.51(t,4H,CH_2),2.98-3.15(sixtet,2H,CH),2.58-2.69(t,4H,CH_2),2.13-2.27(q,4H,CH_2) 1.96-2.02(p,4H,CH_2) 1.45-1.62(q,4H,CH_2),1.23-1.43(m,32H,CH_2),(0.80-1.00,6H,CH_3).

Evaluation of the antibacterial activity of castor oil and its amides

Castor oil and its amide show good activity against gram-positive bacteria (*Staphylococcus aureus*) and gram-negative bacteria (*Pseudomonas aeruginosa*) in different inhibition regions,. The antibacterial activity of castor oil and its amide due to chemical compounds such as phenolic acids, glycosides, alkaloids, flavonoids, and other compounds in oil and amide. These chemical compounds destroy membranes and cell walls of bacteria (SaraJliJa *et al.*, 2012).

It's found that the concentration of the inhibitors is proportional to the diameter of the inhibition regions as shown in the following Table (5):

Table 5 : The biological activity of Gram-positive bacteria (*Staphylococcus aureus*) and Gram-negative (*Pseudomonas aeruginosa*) and the diameter of the inhibition regions (mm) of these bacteria for the at concentrations (200,100, 50) µg/ml.

Compounds	Concentrations µg/mL	*Staphylococcus aureus*	*Pseudomonas aeroginosa*
Castor oil	200	21	20
	100	19	17
	50	---	14
Amide	200	30	20
	100	20	18
	50	20	14

The extracted castor oil prevents the growth of all the tested organisms among gram-positive bacteria, *Staphylococcus aureus* where it gave inhibition areas of 21mm, 19 mm, and ---. For gram negative bacteria *Pseudomonas aeruginosa* where it gave inhibition areas of 20 mm, 17 and 14mm, respectively. This proves that the castor oil has antibacterial activity. For the synthesized amides, it noted that it has prevented to the growth of tested bacteria, both gram-positive and gram-negative, where it noted that the amide has prevent the growth of Gram-positive bacteria and has given inhibiting areas that are (30) mm, (20) and (20) mm. For the gram-negative bacteria, Amide gives inhibiting regions (20) mm, (18) mm and (14) mm. The inhibition activity of castor oil and its amide on *Staphylococcus aureus* and *Pseudomonas aeroginosa* can be illustrated by the following images at a concentration (200 µg/mL):

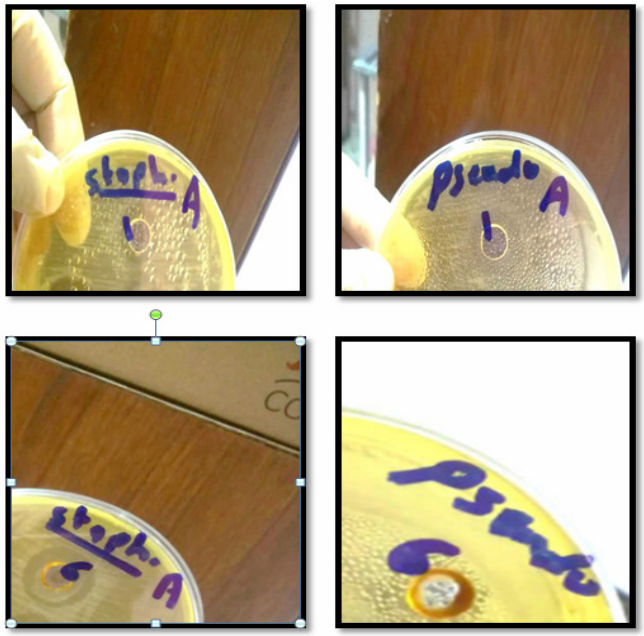


Fig. 5 : Castor oil (1) andAmide (6) against (*Staphylococcus aureus* and *Pseudomonas aeruginosa*) at a concentration (200 µg/mL).

Also, the inhibition activity of castor oil and its amide on *Staphylococcus aureus* and *Pseudomonas aeroginosa* can be shown by the following images at a concentration (100 µg/mL):

Fig. 6 : Castor oil (1) and Amide (6) against (*Staphylococcus aureus* and *Pseudomonas aeruginosa*) at a concentration (100 µg/mL).

The inhibition activity of castor oil and its amide on *Staphylococcus aureus* and *Pseudomonas aeroginosa*, also, can be showed by the following images at a concentration (50 µg/mL):

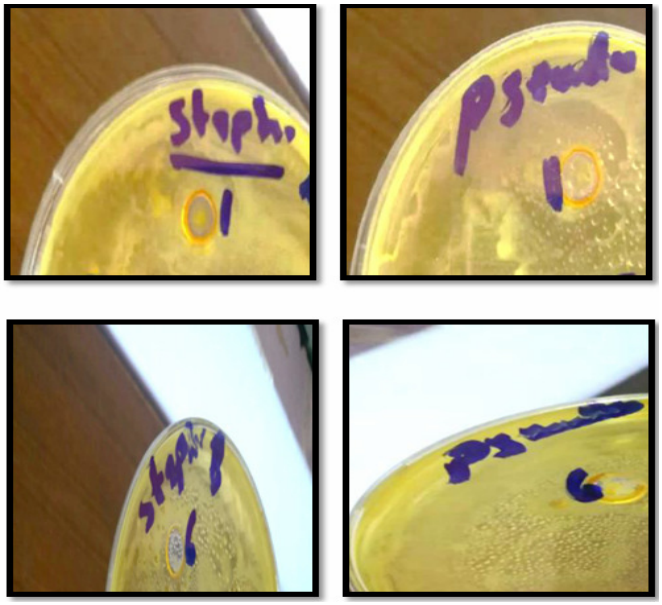


Fig. 7 : Castor oil (1) and Amide (6) against (*Staphylococcus aureus* and *Pseudomonas aeruginosa*) at a concentration (500 µg/mL)

The antibacterial activity of amide due to effective groups in it which obtained from the reaction between ethylene diamine and extracted castor oil (Kadhim *et al.*, 2016).

Conclusion

The inhibitory or bioactivity effect of the oil extract and amide is due to the presence of some organic compounds in the extracts such as phenols, flavonoids, tannins, alkalis, saponins, and phytosterol. Also, the presence of effective groups such as (OH) in the chain and double-bound and the length of the hydrocarbon chain in addition to the presence of nitrogen atoms in the prepared amides.

References

Akpan, U.G.; Jimoh, A. and Mohammed, A.D. (2006) Extraction Characterization and Modification of Castor Seed Oil. Leonardo Journal of Sciences, 8: 43-52.

Al-Sehemi, A.G.; Irfan, A.; Alrumman, S.A. and Hesham, E.A. (2016). Antibacterial activities, DFT and QSAR studies of quinazolinone compounds. Bull. Chem. Soc. Ethiop. 30(2): PP 307-316.

Al-Snafi, A.E. (2017). The pharmacology of Equisetum arvense- A review. IOSR Journal of Pharmacy, 7(2): 31-42.

Al-Snafi, A.E. (2017). Therapeutic and biological activities of *Daphne mucronata* - A review. Indo Am J P Sci, 4(02]: 235-240.

Ambindei, W.A.; Jazet, P.M.D.; Tatsadjieu, L.N.; Priya, P.; Manilal, V.B.; Krishnakumar, B. and Zollo, P.H.A. (2017). Effect of the essential oils of *Thymus vulgaris*, *Cinnamomumzeylanicum* and *Menthapiperita* on fungal growth and morphology. African Journal of Biotechnology, 16(9): 388-399.

Athraa, B.M. *et al.* (2019). Synthesis, Characterization and Biological Activity of New Derivatives of Hetrocyclic Compound. Journal of Global Pharma Technology. 11(08): 146-153.

Auta, M. (2013). Extraction and Characterization of Drilling Oil from Castor Oil. International Journal of Innovation and Applied Studies, 3: 382-387.

De Souza Schneider, R.C.; Baldissarelli, V.Z.; Trombetta, F.; Martinelli, M. and Caramão, E.B. (2004). Optimization of gas chromatographic–mass spectrometric analysis for fatty acids in hydrogenated castor oil obtained by catalytic transfer hydrogenation. Analytica, Chimica, Acta, 505(2): 223-226.

Felipe, A.P.; Andres, A.A.; Herrera, G.; Jose, F.; Vasco-Leal.; Jose D.M.; Beatriz, M.; Mario, E.R. (2013). Physicochemical characterization of seven Mexican *Ricinus communis* L. seeds & oil contents. biomass and bioenergy. 48: PP 17-24.

Hameed, B.H.; Ahmad, A.L. and Ng, A.H. (2003). Removal of residual oil from palm oil mill effluent using solvent extraction method. Jurnal Teknologi, 38(1): 33-42.

Hindi, S.S. and Dawoud, U.M. (2019). Characterization of Hot Pressed-Fixed Oil Extracted from *Ricinus communis* L. Seeds.

Isah, A.G. (2006). Production of detergent from castor oil. Leonardo Electronic Journal of Practices and Technologies, 9: 153-160.

James, A.T.; Hadaway, H.C. and Joan, P.W.W. (1965). The Biosynthesis of Ricinoleic Acid. Biochemical Journal, 95: 448-452.

Jumat, S.; Dina, A.M.N.; Nazrizawati, A.T.; MohdFirdaus, M.Y. and Noraishah, A. (2010). Fatty acid composition and physicochemical properties of Malaysian castor bean *Ricinus communis* L. seed oil. Sains Malaysiana. 39(5): 761-764.

Kadhim, M.J.; Sosa, A.A. and Hameed, I.H. (2016). Evaluation of anti-bacterial activity and bioactive chemical analysis of *Ocimum basilicum* using Fourier transform infrared (FT-IR) and gas chromatography-mass spectrometry (GC-MS) techniques. Journal of pharmacognosy and phytotherapy, 8(6): 127-146.

Kumar, V.S. and Navaratnam, V. (2013). Neem (*Azadirachta indica*): Prehistory to contemporary medicinal uses to humankind. Asian Pacific Journal of Tropical Biomedicine, 3(7): 505–514.

Kyari, M.Z. (2008). Extraction and Characterization of Seed Oils. International Agrophysics, 22: 139-142.

Muzenda, F.; Kabuba, J.; Mdletye, P. and Belaid, M. (2012). Optimization of Process Parameters for Castor Oil Production. Proceedings of the World Congress on Engineering 2012, Vol. III, London, 4-6 July 2012, 1586-1589.

Oliniyan, A.M. (2010). Effect of Extraction Conditions on the Yield and Quality of Oil from Castor Bean. Journal of Cereals and Oilseeds, 1: 24-33.

Omari, A.; Mgani, Q.A. and Mubofu, E.B. (2015). Fatty acid profile and physico-chemical parameters of castor oils in Tanzania. Green and Sustainable Chemistry, 5(04): 154.

Patel, V.R.; Dumancas, G.G.; Viswanath, L.C.K.; Maples, R. and Subong, B.J.J. (2016). Castor oil: properties, uses, and optimization of processing parameters in commercial production. Lipid insights, 9: LPI-S40233.

Salimon, J.; Noor, D.A.M.; Nazrizawati, A.T.; Yusoff, M.F.M. and Noraishah, A. (2010). Fatty acid composition and physicochemical properties of Malaysian castor bean *Ricinus communis* L. seed oil. Sains Malaysiana, 39(5): 761-764.

SaraJliJa, H.; ĈukelJ, N.; Mršić, G.N.D.; Brnĉić, M. and Ćurić, D. (2012). Preparation of flaxseed for lignan determination by gas chromatography-mass spectrometry method. Czech Journal of Food Sciences, 30(1): 45-52.

Tuchilus, C.G.; Nichifor, M.; Mocanu, G. and Stanciu, M.C. (2017). Antimicrobial activity of chemically chemically modified dextran derivatives. Carbohydr Polym. 1(16): 181-186.

Wang, C.; G-r, L.; Z-y, Z.; Peng, M.; Y-s, S.; Luo, R. and Y-s, C. (2013). Genetic diversity of castor bean (*Ricinus communis* L.) in Northeast China revealed by ISSR markers. Biochem. Syst. Eco. (51): 301-307.