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ONE POT SYNTHESIS, CHARACTERIZATION, ANTIMICROBIAL AND ANTI FUNGAL ACTIVITIES OF DIFFERENT FATTY ACID METHYL ESTERS.

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ABSTRACT

Different methyl esters were synthesized using corresponding fatty acids, methanol and hydrogen peroxide as a catalyst along with sulphuric acid. The structure of newly synthesized compounds were confirmed using several spectroscopic techniques such as IR, ^{1}H-NMR, ^{13}C NMR, mass spectral analysis and elemental analysis. The newly synthesized ester derivatives were screened for their *in vitro* antibacterial and antifungal activities. It was observed that some of these compounds had shown promising activity against several bacterial and fungal strains.

KEY WORDS: **Esterification, Hydrogen peroxide, Fatty acids, Antimicrobial activity**

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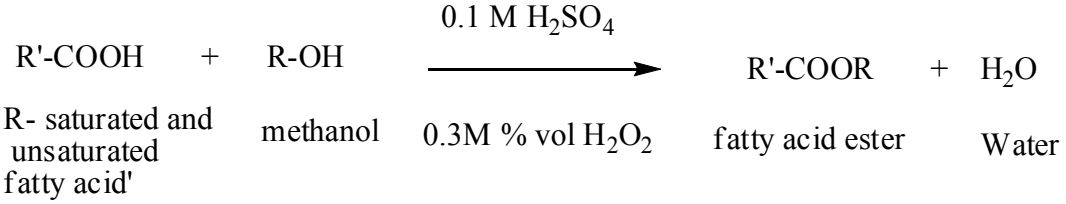
INTRODUCTION

Esterification is one of the most fundamental and important reactions in organic synthesis[1].Although several methods have been explored and developed [2], the use of large amounts of condensing reagents and activators should be avoided in order to promote green chemistry and atom efficiency. The direct condensation of carboxylic acids with alcohols using small amounts of catalysts is the most ideal method but in most cases, large excess amounts of either carboxylic acids or alcohols are used in this condensation to give esters in high yield [3-4].Fatty acids alkyl esters show an increasingly growing demand especially in Europe and the USA due to their numerous applications in cosmetic, pharmaceutical and food industries[5]. These also have considerable demand as bio lubricants for high precision machinery and as bio solvents. In fact, in recent years, concern over the potential impact of petroleum-based solvents on the environment has created an opportunity to promote environmentally acceptable alternatives and bio solvents have been developed as one type of environment friendly product[6]. Several synthesis routes have been reported for the production of fatty acid esters ranging as extraction from natural sources to esterification in the presence of variety of catalysts. Traditionally several synthetic routes are also available for obtaining organic esters [7-10]. Recently several modifications have been under-taken for the preparation of esters in order to minimize lengthy work-ups and to improve yields [11-13]. Different catalyst is also used to give esters in good yield [14-19].Lipids and fatty acids are constituents of all plant cells, where they function as membrane components, storage products and as a source of energy. Fatty acids are widely occurring in natural fats and as in dietary oils. They are also important nutritious substances and metabolites in living organisms[20] linolenic acid and linoleic acid are essential fatty acids for human health[21]. Many fatty acids have bactericidal properties such as oleic, palmitic, stearic, myristic, linoleic and linolenic acids have been demonstrated to have activity against *Clostridium perfringens* and *Streptococcus pyogenes*[22]. Free saturated and unsaturated fatty acids of seaweeds showed anti-tubercular and anti-bacterial activities[23]. The bacteriostatic and bactericidal effects of 30 straight-chain fatty acids and their derivatives on a range of Gram-positive and Gram-negative bacteria were investigated by Kabara[24] Ouattara[25] reported that the fatty acids such as lauric, palmitic and oleic acid possess antibacterial activity. In this paper we described the new method[26] of synthesis of different Fatty acid Esters by using hydrogen peroxide as a catalyst. The aim of our research was to develop an efficient catalytic procedure for the direct esterification of carboxylic acids with alcohols under solvent free conditions with the following requirements:(1) esters should be obtained in high yields by the esterification of carboxylic acids and alcohols (2) esters should be easy to isolate and purify (3) It should involves easy work up, less time, eco friendly side product like water.

EXPERIMENTAL

REACTION SCHEME



MATERIALS

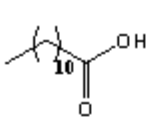
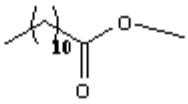
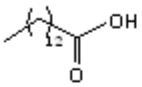
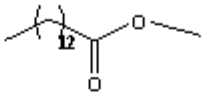
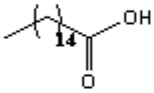
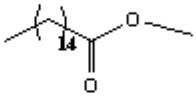
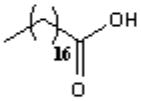
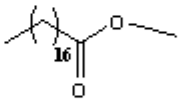
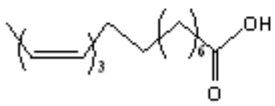
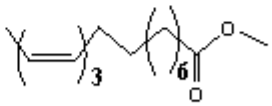
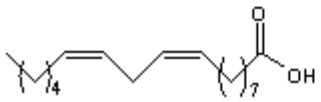
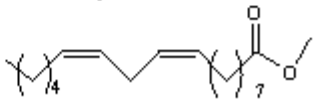
All the chemicals and solvents used were of AR grade. Methanol (Mass fraction purity : 99.9 % E-Merck, India), Lauric Acid (99.9 wt % Merck), Stearic acid (98.9 wt % Merck), (Palmitic acid (98.9 wt % Merck), Myristic acid (98.9 wt % Merck), Linoleic acid (98.9 wt % Merck), Linolenic acids (98.9 wt % Merck), concentrated sulphuric acid ,were used without further purification. Hydrogen peroxide 30% (100 ml by volume) from E-Merck, India was used without further purification.

DETECTION METHODS

The completion of the reaction was continuously monitored by TLC. Melting points taken in open capillaries on TOSHNIWAL melting point apparatus and were uncorrected. IR spectra were recorded on Shimadzu Dr-8031 instrument in KBr pellets. Elemental analysis was carried out using a Perkin-Elmer, CHN elemental analyzer model 2400. ^{1}H-NMR and ^{13}C-NMR spectra of the synthesized compounds were recorded on a Bruker-Avance (300MHz) and Varian-Gemini (200MHz) spectrophotometer using $CDCl_3$ solvent and TMS as an internal standard. EI-MS spectra were determined on a LCQ ion trap mass spectrometer (Thermo Fisher, San Jose, CA, USA), equipped with an EI source.

SYNTHESIS OF FATTY ACIDS METHYL ESTERS (1 a-1 f)

In a typical experimental procedure, the substrate of fatty acid (0.1 M) was dissolved in methanol (0.3 M) to this reaction mixture, H_2SO_4 solution (0.1 M) was added drop wise with constant shaking and cooling followed by slow addition of 0.3 M H_2O_2 solution was added slowly at room temperature. The mixture was refluxed in an oil bath, the completion of the reaction was monitored by TLC. After the completion of reaction it was cooled to room temperature. The crude precipitate obtained was washed 2-3 times with water then with 10% of aqueous $NaHCO_3$ solution to neutralize traces of acids present and then with sufficient volume of distilled water. The product was then dried over anhydrous Na_2SO_4. Table 1 Difference in the % Yield of different fatty acids esters in the presence hydrogen peroxide as a catalyst and absence of hydrogen peroxide (Blank experiment)

Entry	R'-COOH	Time (tlr)	R'-COOR	Yield (%)	With H_2O_2	Blank expt
1a	 Lauric Acids	2	2a	 Methyl Laurate	88%	56%
1b	 Myristic Acid	2	2b	 Methyl Myristate	77%	55%
1c	 Palmitic Acid	2	2c	 Methyl Palmitate	85%	66%
1d	 Stearic Acid	1	2d	 Methyl Stearate	86%	50%
1e	 Linoleic acid	2	2e	 Methyl linoleate	88%	62%
1f	 Linolenic Acid	2	2f	 Methyl Linolenate	86%	64%

BIOLOGICAL ACTIVITY

The newly synthesized compounds were examined for antibacterial and antifungal activity using well diffusion method against the panel of different gram positive and gram negative bacterial strains and fungi strains[27]. Different bacterial strains used for the screening were *S. aureus* (gram positive), *B. subtilis* (gram positive), *P. vulgaris* (gram Negative), and *E. coli* (gram Negative). Antifungal activities of these compounds were also tested against *C. albicans* and *A. niger*. The stock solutions of methyl ester derivatives or standard drug in dimethyl sulfoxide (100 μg/mL) were prepared for the study. The sterilized petri dishes and agar medium were used in the present work. The antibacterial activities of compounds were evaluated by measuring the zone of inhibition on nutrient agar plate. Muller Hinton agar was used in the anti-bacterial study whereas Sabouraud's Dextrose agar was used for the anti-fungal activity study. The composition of nutrient agar medium to culture the bacterial strains used in the present study was as follows: Peptone (10 gm), agar powder (20 gm), sodium chloride powder (10 gm), beaf extract (5 gm), and distilled water (1000 mL). The pH of the nutrient agar medium was adjusted to 7.2. The nutrient agar medium was mixed well and was autoclaved at 15 lbs pressure at 120°C for at least 15 minutes. In the sterilized agar medium, 10mL of one-day-old bacterial/fungal cultures were added. Bacterial or fungal culture were inoculated into nutrient broth and incubated at 37 ± 2°C on a rotary shakerRPM at 100 rpm. After 36-hour incubation, bacterial suspensions were used for further tests. This media were poured in petri dishes and allowed

to set. Two wells were created using a 5mm cork borer. In this well 0.1mL of test sample/standards were filled. All the 5 nutrient agar plates were incubated at 37°C for 24 hours in anti-bacterial study and at 37°C for 48 hours in anti-fungal activity study. The plates were observed for clear zone of inhibition. Then diameters of the zone of inhibition for these compounds were measured. The biological activities were tested for at least three times for all the compounds against all microorganisms and average value has been reported here. The results of antimicrobial activity and antifungal activity of the test compounds have been collected in Table II.

RESULTS AND DISCUSSION

To evaluate the potential of hydrogen peroxide as a catalyst, we initially examined its use for the synthesis of methyl esters of different fatty acids. A comparative esterification study was carried out in the presence and absence of hydrogen peroxide (blank experiment) in Table. 1. It has been found that % yield of 2a (88%), 2b (77%), 2c (85%), 2d (77%), 2e (88%), 2f (86%) ester produced by the use of hydrogen peroxide as a catalyst is increased drastically as compared to that in absence of hydrogen peroxide 2a' (56%), 2b' (55%), 2c' (66%), 2d' (50%), 2e' (62%), 2f' (64%) respectively. The newly synthesized fatty acids methyl esters were characterized on the basis of their spectroscopic data (^{1}H-NMR, ^{13}C-NMR, IR and MASS) and elemental analysis. In the elemental analysis, the percentage of the nitrogen, hydrogen and carbon found to be experimentally equivalent to the calculated values in all the compounds. In general, compounds 3a-3f have shown C=O peak of ester group within the range of 1730-1750cm^{-1}, the ^{1}H-NMR spectra have shown the peaks of protons present in the compounds at their appropriate δ values whereas ^{13}C- NMR confirmed the numbers of C atoms in the synthesized compounds. In the LCMS of synthesized, peaks for molecular ion peaks were observed at its respective molecular in mass. The newly synthesized esters we evaluated for in vitro antibacterial and antifungal activities against various gram positive and gram negative bacterial and fungal species. The result have been collected in Table 2. Standard Ciprofloxacin were tested against bacterial strains were as Flucanzole was tested against fungal strains for comparison with newly synthesized compounds. Lauric acid possesses antibacterial activity supports present study. Similarly long chain unsaturated fatty acids, including linoleic acids are well known to inhibit bacteria like *E-coli.* Long chain fatty acids have higher antimicrobial activity against gram positive bacteria than gram -negative bacteria

Table 2

Anti bacterial and Anti fungal Activities of Different Fatty Acid Methyl Esters

Compounds (500µg/disk)	Average value of zone of inhibition in mm					
	Anti bacterial Activity				Anti fungal Activity	
	Gram Positive		Gram Negative			
	S. aureus	*B. subtilis*	*P. vulgaris*	*E. Coli*	*C. albican*	*A. nigar*
3a	20.3 ±1.5	20.5 ±1.3	13.8 ±1.0	12 ± 0.8	18.5 ±1.3	12.3 ±0.5
3b	18.5±1.3	18.5 ±1.3	12.5 ± 1.0	11 ± 0.8	12±0.8	7.8± 0.5
3c	17.5±1.3	17.3±1.3	12.3 ±1.0	10.5 ±0.6	14.3 ± 1.0	7.8 ±0.5
3d	17.5 ±1.3	17.3 ±1.3	12.3 ±1.0	10.5 ±0.6	16.5 ± 1.0	7.8 ±0.5
3e	18.5 ±1.3	18.3 ±1.3	12.8 ± 1.0	12 ±0.8	10.3± 1.0	11.5 ±0.5
3f	17.5 ±1.3	17.3 ± 1.3	12.3 ±1.0	10.5 ±0.6	12.3 ±1.0	10.5 ± 0.5
Ciprofloxacin	24	22	20	20	------	--------
Fluconazole	----	------	-----	------	20	20

CHARACTERIZATION DATA OF METHYL LAURATE

(2a) Colorless liquid; Yield 87.85%; B.P 148^{o}C; IR 1744 (KBr) cm^{-1} (carboxylic ester group C=O); ^{1}H –NMR ($CDCl_3$) δ,3.65 (s, 3H), 2.32 (t, 2H), 1.64 (m, 2H), 1.29 (m, 2H), 1.26 (m, 2H), 1.26 (m, 2H), 1.26 (m, 2H), 1.26 (m, 2H), 1.29 (m, 2H), 1.29 (m, 2H), 1.31 (m, 2H), 0.88 (t, 3H);^{13}C-NMR (100MHz, $CDCl_3$ d_6, δ, ppm) : 173.1, 51.9, 33.6, 25.0, 29.0, 29.3, 29.6, 29.6. 29.6, 29.3, 31.8, 22.7, 14.1 (13 aliphatic carbon); Elemental analysis for $C_{13}H_{26}O_2$ (%): C 72.84, H 12.33, O 14.93; Found (%): C 72.62, H 12.20, O 14.85; Mass; m/z 214.19 (100%, M^+),199, 185, 171, 157, 143, 129, 115, 101, 87, 74, 55.

CHARACTERIZATION DATA OF METHYL MYRISTATE

(2b) Colorless liquid; Yield 70.95 %; B.P 148^{o}C; IR 1736 (KBr) cm^{-1} (carboxylic ester group C=O) ;^{1}H-NMR ($CDCl_3$) δ, 3.65 (s, 3H), 2.32 (t, 2H), 1.64 (m, 2H), 1.29 (m, 2H), 1.26 (m, 2H), 1.26 (m, 2H), 1.26 (m, 2H), 1.26 (m, 2H),1.26 (m, 2H) , 1.26 (m, 2H), 1.29 (m, 2H), 1.29 (m, 2H), 1.31 (m, 2H), 0.88 (t, 3H); ^{13}C-NMR (100MHz,$CDCl_3$ d_6, δ, ppm) :173.1, 51.9, 33.6, 25.0, 29.0, 29.6, 29.6. 29.6, 29.6, 29.6, 29.3, 31.8, 22.7, 14.1 (15 aliphatic carbon); Elemental analysis for $C_{15}H_{30}O_2$ (%): C 74.32, H 12.47, O 13.20; Found (%): C 74.28, H 12.50, O 13.22; Mass, m/z 242.22 (100 %, M^+): 227, 213, 99, 185, 171, 157, 143, 129, 115, 101, 87, 74, 55

CHARACTERIZATION DATA OF METHYL PALMITATE

(2c) Colorless liquid; Yield 70.95%; B.P 164^{o}C; IR 1743 (KBr) cm^{-1} (carboxylic ester group C=O) ^{1}H NMR ($CDCl_3$) δ,3.65 (s, 3H), 2.32 (t, 2H), 1.64 (m, 2H), 1.29 (m, 2H), 1.26 (m, 2H), 1.26 (m, 2H), 1.26 (m, 2H), 1.26 (m, 2H), 1.26 (m, 2H), 1.26 (m, 2H), 1.26 (m, 2H), 1.26 (m, 2H), 1.29 (m, 2H), 1.29 (m, 2H), 1.31 (m, 2H), 0.88 (t, 3H) ; ^{13}C NMR (100MHz, $CDCl_3$ d_6, δ, ppm) :173.1, 51.9, 33.6, 25.0, 29.0, 29.3, 29.6, 29.6. 29.6, 29.6. 29.6, 29.6. 29.6, 29.3, 31.8, 22.7, 14.1 (17 aliphatic carbon); Elemental analysis for $C_{17}H_{34}O_2$ (%): C 75.50, H 12.67, O 11.83; Found (%): C 75.22, H 12.05, O 14.70; Mass; m/z 270 (100%, M^+), 241, 239 [M-31$^+$], 227. 213, 199, 185, 171, 157, 143, 129, 115, 101, 87, 74, 55

CHARACTERIZATION DATA OF METHYL STEARATE

(2d) Crystalline solid; Yield 81.87%; M.P 39^{o}C; IR 1743 (K Br) cm^{-1} (carboxylic ester group C=O) ^{1}H NMR ($CDCl_3$) δ: 3.65 (s, 3H), 2.32 (t, 2H), 1.64(m, 2H), 1.29 (m, 2H), 1.26 (m, 2H), 1.26 (m, 2H), 1.26 (m, 2H), 1.26 (m, 2H), 1.26 (m, 2H), 1.26 (m, 2H), 1.26 (m, 2H), 1.26 (m, 2H), 1.26 (m, 2H), 1.26 (m, 2H), 1.29 (m, 2H), 1.29 (m, 2H), 1.31 (m, 2H), 0.88 (t, 3H), ^{13}C NMR (100 MHz,$CDCl_3$ d_6, δ, ppm) :173.1, 51.9, 33.6, 25.0, 29.0, 29.3, 29.6, 29.6. 29.6, 29.6. 29.6, 29.6. 29.6, 29.6. 29.6, 29.3, 31.8, 22.7, 14.1 (19 aliphatic carbon); Elemental analysis for $C_{19}H_{38}O_2$ (%): C 76.45, H 12.83, O 10.72; Found (%): C 76.42, H 12.82, O 10.66; Mass; m/z 270 (100%, M^+), 241, 239 [M-31$^+$], 227. 213, 199, 185, 171, 157, 143, 129, 115, 101, 87, 74, 55

CHARACTERIZATION DATA OF METHYL LINOLEATE

(2e) Clear liquid; Yield 80.24%; B.P 192^{o}C; IR 1744 (KBr) cm^{-1} (carboxylic ester group C=O) ^{1}H-NMR ($CDCl_3$) δ: 3.65 (s, 3H): 2.32 (t, 2H), 2.63 (m, 2H), 2.18 (m, 2H), 2.18 (m, 2H), 1.64 (m, 2H), 1.29 (q, 2H), 1.29 (q, 2H), 1.29 (m, 2H), 1.29 (m, 2H), 1.29(m, 2H), 1.29(m, 2H), 1.31(m, 2H), 5.49 (m, 1H -CC=C cis), 5.49 (m, 1H -CC=C cis), 5.40 (m,1H -CC=C cis), 5.40(m, H -CC=C cis; 0.90(t, 3H); ^{13}C NMR (100 MHz,$CDCl_3$ d_6, δ, ppm) :173.1, 51.9, 127.3, 127.3, 127.3, 130.3, 130.3, 33.6, 37.6, 33.6, 33. 8, 33. 8, 25.0, 29.9, 29.6, 29.0. 29.7, 29.4. 31.9, 22.8, 14.1(19 aliphatic carbon); Elemental analysis for $C_{19}H_{34}O_2$ (%): C 77.50, H 11.64, O 10.87; Found (%): C 77.46, H 11.69, O 10.85; Mass; m/z 294(100%, M^+),263 [M-31$^+$], 252,237.220[M-74], 205, 191, 178, 164, 150, 136, 123, 109, 95, 81, 74, 67, 55.

CHARACTERIZATION DATA OF METHYL LINOLENATE

(2f) Clear liquid; Yield 70.50%; B.P 182^{o}C; IR 1742 (KBr) cm^{-1} (carboxylic ester group C=O) ^{1}H NMR ($CDCl_3$) δ:3.65 (s, 3H), 2.32 (t, 2H), 2.63 (m, 2H), 2.63 (m, 2H), 2.18(m,

2H), 2.00 (m, 2H), 1.64 (m, 2H), 1.29 (m, 2H), 1.29 (m, 2H), 1.29 (m, 2H), 1.29 (m, 2H), 1.06 (m, 2H), H 5.38 (m, 1H,CC=C trans), 5.38 (m, 1H ,CC=C trans), 5.43 (m, 1H ,CC=C trans), 5.43(m, 1H ,CC=C trans), 5.37 (m, 1H-CC=C trans),5.37 (m, 1H-CC=C trans) ;^{13}C NMR (100 MHz,$CDCl_3$ d_6, δ ppm) :173.1, 51.9, 127.3, 33.6, 25.1, 29.1, 29.5, 29.8, 30.0, 27.8, 132.2, 25.7, 128.8, 128.8, 25.7, 128.0, 14.3, 128.9, 20.6 (19 aliphatic carbon); Elemental analysis for $C_{19}H_{32}O_2$ (%): C 78.03, H 11.03, O 10.94; Found (%): C 78.05, H 11.08, O 10.87; Mass ;m/z 292(100%, M^+), 261 [M-31$^+$], 249, 236. 223[M-74], 203, 191, 173, 163, 149, 135, 121, 108, 95, 79, 67, 55.

CONCLUSION

The key advantages of this method are the simplicity of work up, ease and safety of reagents handling, cheapness of catalyst and no organic by product which must be isolated from product after reactions. A series of different fatty acids methyl esters were successfully synthesized. All the esters prepared by using hydrogen peroxide in one pot synthesis were in good yield. They were characterized by spectroscopic ally and using analytical techniques such as ^{1}H-NMR, ^{13}C-NMR, IR, and MASS and elemental analysis. Antibacterial activity studies reveals that compounds 2a and 2e showed highest activity among all the synthesized esters as compared to standard drug, where as compounds 2a have shown the highest antifungal activity among all newly synthesized compounds as compared to standard drug.

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