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PHENOLIC ACID DERIVATIVES AS PRESERVATIVES: SYNTHESIS, ANTIOXIDANT, ANTIMICROBIAL POTENTIAL AND THEIR PRESERVATIVE EFFECTIVENESS

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ABSTRACT: Natural phenolic acids alkyl esters *viz* methyl, ethyl, propyl was synthesized and characterized by spectral means FTIR, 1HNMR, and 13CNMR. All the synthesized esters were examined for their antimicrobial potential, preservative efficacy and antioxidant potential. Among the synthesized ester derivative caffeic acid and gallic acid, derivatives were displayed excellent antioxidant and antimicrobial potential. They were further evaluated their preservative efficacy according to USP 2004 protocol for preservative effectiveness testing. Caffeic acid propyl ester and gallic acid ethyl ester exhibited promising preservative potential better than existing preservative agents. From the study, we can conclude that these caffeic and gallic acid derivatives can be used as lead compounds to further explore their application as preservative agents in pharmaceuticals and in the food industry.

INTRODUCTION: Natural phenolic acids are widely distributed in plants, generally found as amides, glycosidic conjugates, and esters in various fruits and vegetable cell walls [1]. Alkaline hydrolysis is used to extract these phenolic acids from nature. Phenolic acids are widely utilized antioxidants among food, pharmaceuticals, and beauty care products. Numerous scientific studies have reported biological activities of phenolic acids, and most of the studies confirmed that these phenolic acids possessed excellent antitumor, hypoglycaemic, antihypertensive, anti-inflammatory potential *etc.* [2].

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Biological properties of phenolic acids were conferred by benzene ring, carboxylic group, and the molecule structure bearing one or more hydroxyl and/or methoxyl groupings [4]. Formation of resonance stabilized phenoxy radical attributed to the presence of side chain conjugation and phenolic nucleus upon UV absorption phenolic acids promotes the stable radical formation. Hence, their ability is enhanced to terminate free radical chain reactions.

Due to their ability to suppress radiation-induced oxidation reactions and excellent free radical scavenging potential various phenolic acids are being used to protect the physiological integrity of cells. Due to photoprotection action, phenolic acids are incorporated in many cosmetic lotions. The addition of natural phenolic acids in food products increases shelf life by preventing oxidation spoilage by inhibiting lipid peroxidation. By the same mechanism, phenolic acid may protect

against various inflammatory diseases [5]. According to the latest findings, position and a number of free phenolic hydroxyl groups significantly affect the strength of biological activities [6].

Natural phenolic acids have also been reported for their different biological activities, as represented in **Table 1**. Caffeic acid, which is a natural phenolic acid exhibits excellent antioxidant activity [7] and has been reported for its anti-inflammatory, antibacterial and anti-tumor activities [8]. Another natural cinnamic acid: sinapic acid extracted from mustard seeds, was tested against *E. coli* (Gram-negative) and *S. aureus* (Gram-positive) and found to exhibit antimicrobial activity [9]. Phenolic compounds are natural antioxidants widely distributed in plants and become a vital part of the human diet with decreased risk of cardiovascular and cancer diseases linked with oxidative stress [10]. The possession of excellent biological properties of phenolic compounds has become an incentive for scientists to explore these natural compounds further, and it seems logical due to their availability in natural sources. Thus to extend their role in the pharmaceutical and food industries, the solubility and stability of these natural antioxidants must be improved [11]. An amphiphilic molecule can be obtained by esterification of carboxylic acid functional group with alcohols [12]. Moreover, the relationship between their antioxidant efficacy and hydrophobicity is well described by some studies [13]. The present study has reported the synthesis, antimicrobial and antioxidant activity of the synthesized derivatives of phenolic acids along with the preservative efficacy of ethyl 3, 4, 5-trihydroxybenzoate, and propyl 3-(3,4-dihydroxyphenyl) acrylate.

TABLE 1 BIOLOGICAL PROPERTY OF NATURAL PHENOLIC ACIDS

S. no.	Phenolic acid	Bioactivity	Reference(s)
1	Vanillic acid	Anti-cancer, Antimicrobial, Anti-tumor	3
2	Veratric acid	Antitumor, Antihypertensive, Anti-inflammatory potential	14
3	Gallic acid	Anti-bacterial	15
4	Syringic acid	Anti-tumor, Anti-bacterial	16
6	Sinapic acid	Antimicrobial,	12
7	Ferulic acid	Anticancer, Antihypertension, Reducing type 2-diabetes	17
8	Coumaric acid	Anticancer, Anti-inflammation,	18
9	Gentisic acid	Anti-oxidant	19
10	Caffeic acid	Anti-mutagenic and anticancer activity	20
11	Anisic acid	Anti-fungal, Anti-bacterial	21

MATERIALS AND METHODS: All the required chemicals for the synthesis of phenolic acid drivatives and their antimicrobial, antioxidant, and preservative efficacy test were bought from CDH Pvt. Ltd. (New Delhi, India) and Loba Chemie Pvt. Ltd. (Mumbai, India). The reaction monitoring was done by thin-layer chromatography on pre-coated silica gel plates for TLC obtained from Merck (Mumbai, India) and was inspected in UV chambers, Hexane: Ethyl acetate (7:3) was utilized as a solvent for TLC. Sonar melting point apparatus was used to record the melting points.

KBr pellets procedure was used to record Infrared (IR) spectra using Perkin Elmer spectrum II FTIR spectrophotometer. 13C NMR and 1H NMR spectra were affirmed in deuterated CDCl3, and DMSO separated at 400 MHz downfield using tetramethylsilane standard on Bruker Advance II 400 NMR spectrometer. Coupling constants (J) and chemical shifts were recorded in Hertz (Hz) and in parts per million respectively.

Procedure for the Synthesis of Phenolic Acid Chlorides: The selected phenolic acid (20mmol) was stirred at 80 °C for 2-4 h with 10ml thionyl chloride in presence diethyl ether as solvent and pyridine as catalyst. The excess of thionyl chloride was distilled off and the corresponding product was dried and weight as acid chloride. The single spot TLC under UV light confirmed the purity of acid chloride formed.

General Procedure for the Synthesis of Esters of Phenolic Acids Esters: Phenolic acid esters were synthesised by refluxing respective alcohols with the corresponding synthesised acid chlorides (0.05 mol) in 50 ml ether for 8-10 h at 70-80 °C as per Scheme 1, 2 and 3.

The reaction mixture was subjected to reflux on the water bath until the liberation of HCl gas was ceased, and the end of the reaction was confirmed with the appearance of single spot TLC. At the final stage, the ester was extracted with ether, and recrystallization was done with acetone. The percentage yield was recorded at room temperature after drying.

***In-vitro* Antimicrobial Activity:** The prepared esters were examined for antimicrobial susceptibility using the tube dilution method against bacterial strains *K. pneumoniae, E. coli, P. mirabilis, S. aureus, P. Aeruginosa,* and fungal strains *A. niger,* and *C. albicans*. Stock standards of antibiotics *viz.* streptomycin and fluconazole were obtained as gift samples from pharmaceutical companies. The synthesized compounds were dissolved in DMSO (Dimethyl sulfoxide) to a concentration of 100 µg/mL and diluted further to concentrations of 50, 25, 12.5, 6.25, 3.125, and 1.562 µg/mL [22].

Double strength nutrient broth media I.P. for antibacterial study and Sabouraud dextrose broth media I.P. for the antifungal study were used [24]. The test tubes were examined after 24 h of incubation at 37±1°C for bacterial stains and after 2 days of incubation at 25±1°C for *C. albicans* and after 7 days of incubation for *A. niger*. Tubes were scanned for any visible turbidity or sediment, and tubes with no visible growth at least amount of test compound were reported as MIC (Minimal Inhibitory Concentration).

Preservative Efficacy: The selected most active antimicrobial compounds were tested for their preservative efficacy where the *E. coli, P. aeruginosa, S. aureus, C. albicans, and A. nigerwere* used as challenge microorganisms. The results were noted on 14th and 28th day. Pulp-based slurry of cellulose was used to evaluate the preservative effectiveness of synthesized compounds. The test compound and standard preservative compounds were incorporated by adding 0.5% of the test compound and standard compound in a mixture of 100 mg cellulose in 2 mL of 0.1M sodium phosphate buffer and 4 mL of dimethyl sulfoxide [23]. After 24 h stirring of the above mixture at 25 °C the sample was mixed with 1.0 M sodium chloride for 24h and washed with distilled water. The washing was done with DMSO and diethyl ether and distilled water in case of antifungal compounds, and the preparations were stored in sterile containers [24]. Preservative efficacy testing was performed according to the standard protocol as per USP-2004. The inoculum was prepared from recently grown stock culture on agar plates. The sterile test tubes with 10 mL of cellulose slurry were incubated with an inoculum of microorganisms at an optimal temperature under aseptic conditions. Each test tube was scanned at 7, 14, 21, and 28 days to determine the CFU by using the plate count procedure. The number of CFU was noted for each sample and calculated as logarithm values of the number of CFU/mL compared according to the standard protocol of USP 2004. Criteria for the test include not less than 1.0 log reduction from the initial count at 14 days and no increase from the 14 days count at 28th days [25].

Anti-oxidant Activity: DPPH free radical procedure depends on the movement that turns violet coloration in ethanol. Antioxidant action depended on free radical scavenging potential towards stable DPPH radical on spectrophotometric reaction as per the technique detailed in literature [26]. Solutions of synthesized esters in methanol were made in 50 ml quantity at 25, 50, 75, and 100 µg/mL concentrations. A stock solution was prepared by 0.1 mM DPPH dissolved in methyl alcohol and 1 mL from this solution was added to 3 mL of test or standard and incubated at 30 °C for 30 min in the dark. The ascorbic acid was used for differentiation or as a positive control. At first, the clear range for ethanol/water was recorded in this manner; the spectrophotometric titration was done at different concentrations of synthesized compounds, and absorbance was estimated at 517 nm. Absorbance at a lower wavelength of the reaction mixture demonstrated higher free radical scavenging potential. The tests were performed in triplicate, and IC_{50} values were determined by utilizing the equation:

$$IC_{50} = (Ac\text{-}As) \times 100/Ac$$

Here, as is the absorbance of the sample, and Ac is the absorbance of the control

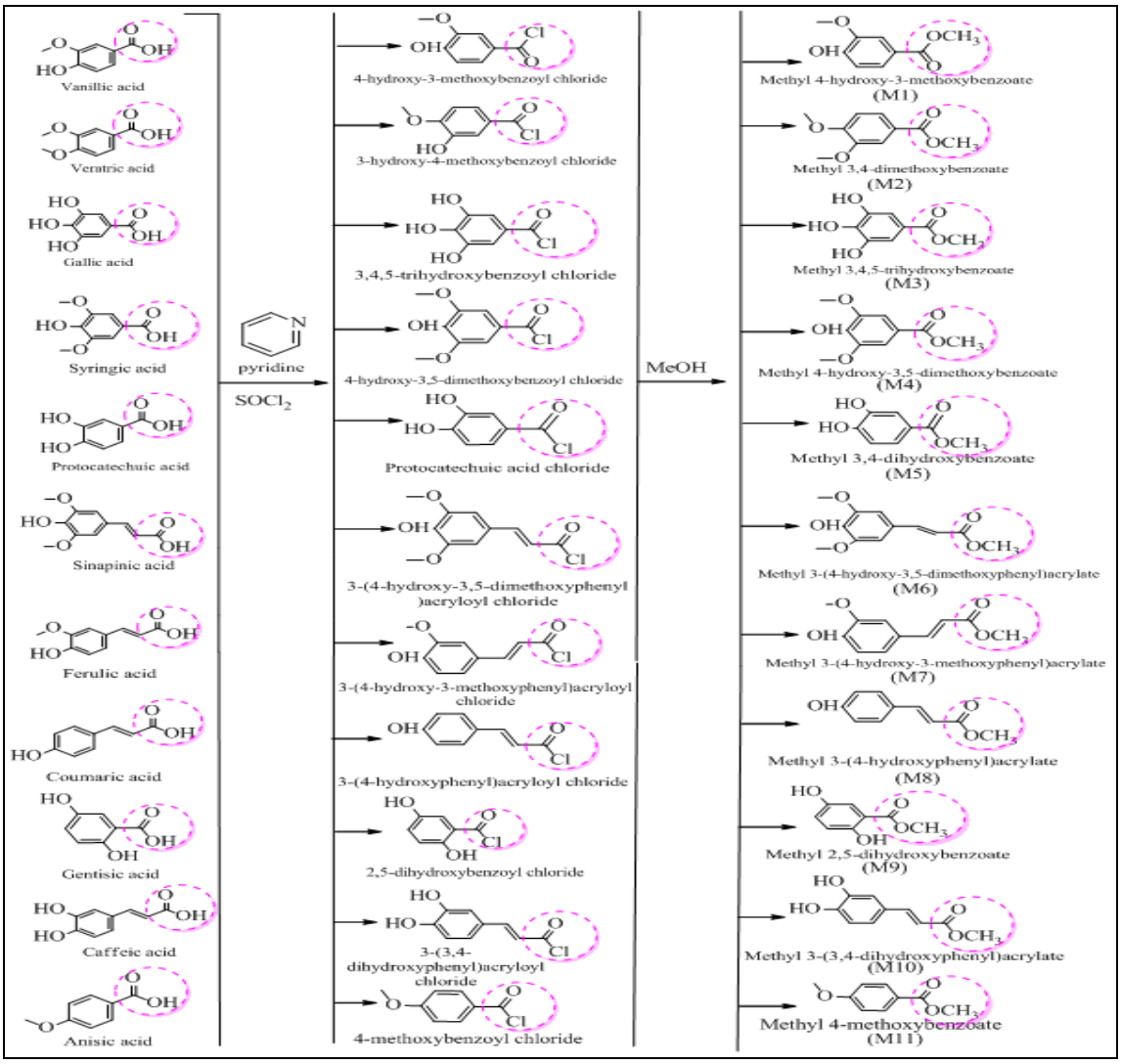
RESULTS AND DISCUSSION:

Chemistry: A series of esters of cinnamic acid and benzoic acid derivatives were synthesised. The

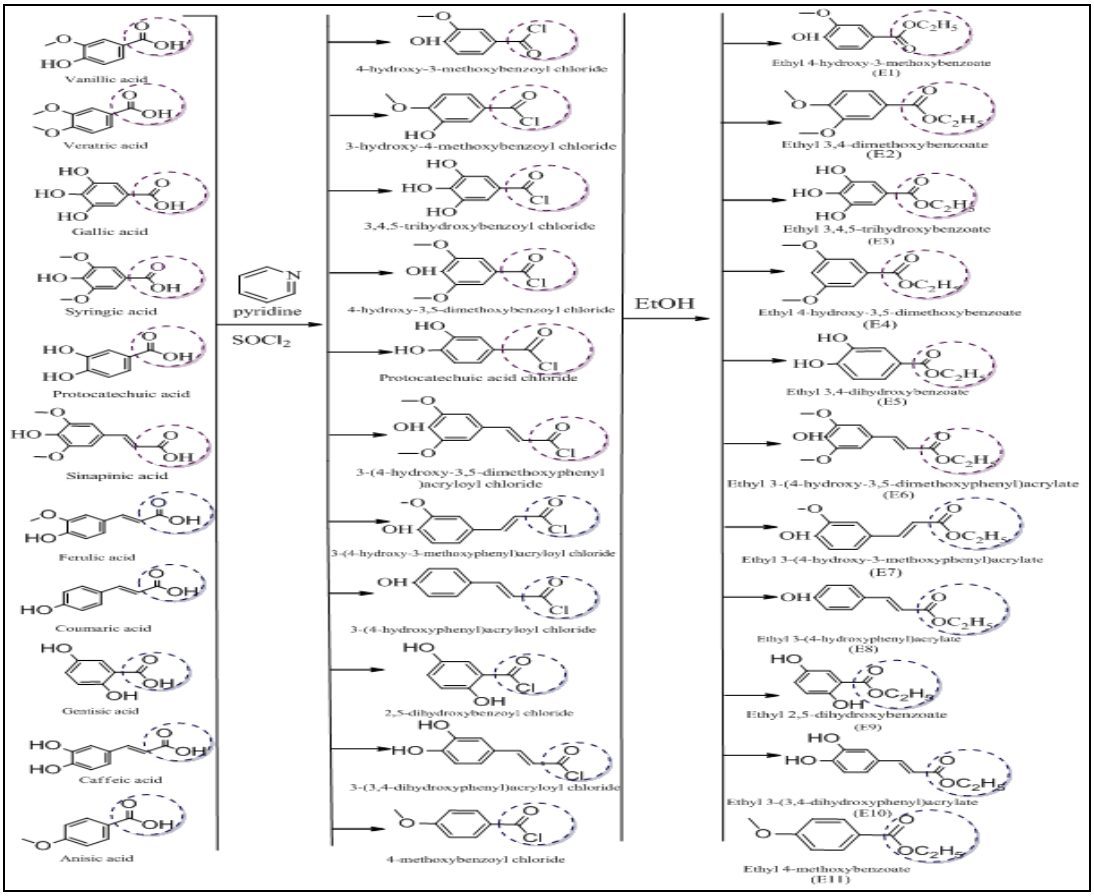
methyl, ethyl, and propyl esters were synthesized per the reaction outlined in Scheme 1, 2 and 3. The final structures of all the synthesized compounds were confirmed using IR, 1HNMR, 13CNMR and were found in close conformity with their molecular structures. The phenolic acid chlorides were initially prepared by stirring the respective phenolic acids (20 mmol) with 10ml thionyl chloride at 80°C for 1-4 h in the presence of diethyl ether as solvent using pyridine as a catalyst.

The reaction completion was examined by thin-layer chromatography (TLC). The single spot TLC under UV light observation confirmed the purity of acid chloride formed. Alkyl esters of phenolic acids were further synthesized by refluxing with the respective alcohols with corresponding acid chlorides (0.05 mol) in ether (50 ml) for 8–10 h at 70-80 °C. The reaction mixture was refluxed, and the end of the reaction was affirmed by single spot TLC. Finally, the ester was extracted with ether, and recrystallization was done with acetone to obtain the final product. Physicochemical properties and spectral data of the synthesized compounds have been shown in **Table 2.** Further, the synthesized products were studied for their spectral data *viz*. FTIR, 1H-NMR, 13C-NMR, wherein the shift in FTIR peaks from 1700 cm^{-1} in phenolic acids for carbonyl group to 1730-1745 cm^{-1} confirmed the corresponding formation ester derivatives.

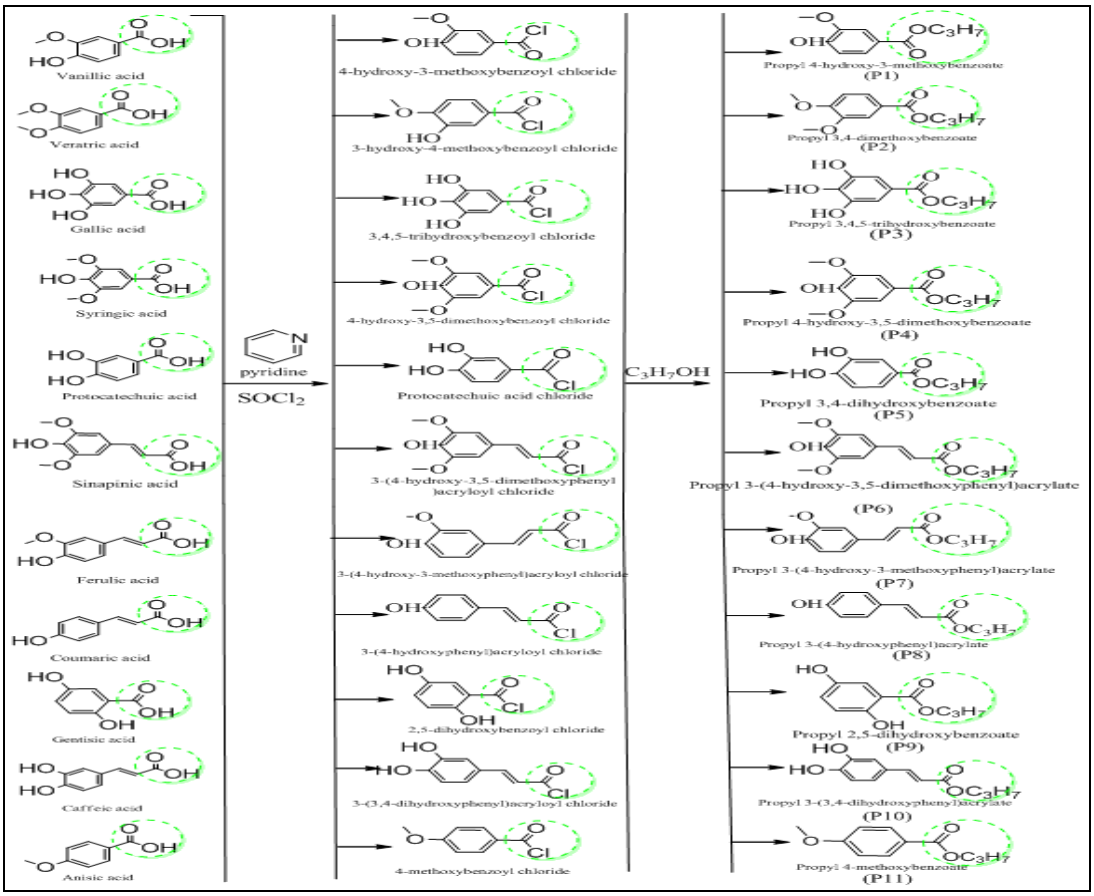
1H-NMR was performed for the above-synthesized structures, and the appearance of chemical shift near δ 4.1-4.6 ppm for the ester linkage and disappearance of the peak near δ 11 ppm for OH functional group of carboxylic acid confirmed the formation of esters of the given phenolic acids. The 13CNMR ester peaks were recorded at 165-180 ppm for synthesized ester derivatives.



SCHEME 1: SYNTHESIS OF METHYL ESTERS



SCHEME 2: SYNTHESIS OF ETHYL ESTERS



SCHEME 3: SYNTHESIS OF PROPYL ESTERS

TABLE 2: PHYSICOCHEMICAL PROPERTIES AND SPECTRAL DATA OF SYNTHESIZED COMPOUNDS

Com. 1	UPAC	M.P (°C)	Rf Value	Spectral details					
				IR (KBr)				1HNMR (400 MHz, DMSO-d6)	13CNMR
				OH str	C_H str	C=O ester	C=C aromatic		
M1	Methyl 4-hydroxy-3-methoxybenzoate	131-133	0.50	3403	2364	1742	1258	7.41 (m, 3H, ArH), 3.94(s, 1H, OH of phenolic hydroxyl), 3.65 (s, 3H, OCH_3,ester)	102, 108,112, 133, 135, 137 (6C, phenyl nucleus), 39.5 (OCH_3), 155.4 (C=O of $COCH_3$)
M2	Methyl 3,4-dimethoxybenzoate	168-170	0.63	3394	2345	1738	1287	^{1}H NMR (400 MHz, DMSO-*d*6) δ: 6.81 (m, 3H, ArH), 3.67(s, 1H, OH of phenolic hydroxyl), 3.67 (s, 3H, -OCH_3,ester)	110.11, 114, 122.32, 122.87, 149.12, 154.89 (6c, phenyl nucleus), 57.23, 56.86 (2C,OCH_3), 162.55 (C=O, $COCH_3$)
M3	Methyl 3,4,5 -trihydroxybenzoate	309-311	0.42	3349	2363	1735	1286	7.29 (m, 3H, ArH), 4.71(s, 1H, OH of phenolic hydroxyl), 3.83 (s, 3H, -OCH_3, ester)	109, 113, 124, 137, 143, 146 (6C, phenyl nucleus), 163 (C=O, $COCH_3$), 53.65 (1C, OCH_3)
M4	Methyl 4-hydroxy-3,5-dimethoxybenzoate	173-175	0.48	3315	2345	1738	1339	7.32 (m, 3H, ArH), 3.93 (s, 3H, -OCH_3, ester)	106, 109, 125, 142, 146, 147 (6C, phenyl nucleus), 165 (C=O, $COCH_3$), 52.29 (1C, OCH_3), 55.81, 54.97 (2C,OCH_3)
M5	Methyl 3,4-dihydroxybenzoate	194-196	0.42	3400	2364	1731	1441	7.44 (m, 3H, ArH), 4.71(s, 1H, OH of phenolic hydroxyl), 3.29 (s, 3H, -OCH_3, ester)	116.72, 118.21, 123.89, 128.78, 144, 151.13 (6C, phenyl nucleus), 163.25 (C=O, $COCH_3$)53.19 (1C, OCH_3)
M6	Methyl 3-(4-hydroxy-3,5-dimethoxyphenyl)acrylate	197-199	0.52	3416	2370	1742	1459	7.31 (m, 3H, ArH), 3.69(s, 1H, OH of phenolic hydroxyl), 3.39 (s, 3H, -OCH_3, ester)	107.34, 109.81, 138.90, 142.56, 147.89, 146.72 (6C, phenyl nucleus), 164.45 ((C=O, $COCH_3$), 53.32 (2C, OCH_3), 56.71 (1C, OCH_3)
M7	Methyl 3-(4-hydroxy-3-methoxyphenyl)acrylate	146-148	0.48	3422	2974	1744	1272	3422 (OH str., phenol), 2974 (C-H str., OCH_3), 1744 (C=O str., ester), 1272 (C=C str., aromatic)	7.80 (m, 3H, ArH), 3.86(s, 1H, OH of phenolic hydroxyl), 3.47 (s, 3H, -OCH_3, ester)
M8	Methyl 3-(4-hydroxyphenyl)acrylate	102-104	0.37	3349	2363	1735	1286	7.29 (m, 3H, ArH), 4.71(s, 1H, OH of phenolic hydroxyl), 3.83 (s, 3H, -OCH_3, ester)	115.45, 129.34, 145.56, 132.12, 134.56, 154.34 (6C, phenyl nucleus), 112.32, 148.65 (2C, C=C), 164.78(C=O, COCH3), 53.17 ((1C , OCH3)
M9	Methyl 2,5-dihydroxybenzoate	195-197	0.42	3421	2362	1745	1247	8.01 (m, 3H, ArH), 4.65 (s, 1H, OH of phenolic hydroxyl), 3.81 (s, 3H, -OCH_3, ester)	113.12, 115.87, 123.77, 127.34, 151.45, 156.25 (6C, phenyl nucleus), 171.35 ((C=O, COCH3), 54.28 ((1C , OCH3);
M10	Methyl 3-(3,4-dihydroxyphenyl)acrylate	212-214	0.38	3368	2362	1732	1258	6.84 (m, 3H, ArH), 4.58 (s, 1H, OH of phenolic hydroxyl), 3.66(s, 3H, -OCH_3, ester)	117.45, 123.34, 131.56, 136.12, 141.75, 147.32 (6C, phenyl nucleus),

									118.34, 142.14 (2C, C=C), 167.47(C=O, COCH3), 55.30 ((1C , OCH3)
M11	Methyl 4-methoxybenzoate	120-122	0.50	3424	2928	1738	1292	7.38 (m, 3H, ArH), 4.72 (s, 1H, OH of phenolic hydroxyl), 3.60 (s, 3H, -OCH_3, ester)	114.65, 120.21, 128.56, 133.12, 137.79, 162.88 (6C, phenyl nucleus), 163.22(C=O, COCH3), 53.47, 57.12 (2C , OCH3)
E1	Ethyl 4-hydroxy-3-methoxybenzoate	143-145	0.48	3421	2362	1749	1247	7.58 (m, 3H, ArH), 3.51 (s, 3H, OCH_3), 3.17 (m, 2H, CH_2 of C_2H_5,ester), 1.25 (m, 3H, CH_3 ofC_2H_5)	113.21, 114.39, 125.12, 127.29, 148.37, 156.28 (6c, phenyl nucleus), 54.35 (1C,OCH3), 162.55 (C=O, COC2H5), 59.92, 14.89 (2C, OC_2H_5)
E2	Ethyl 3,4-dimethoxybenzoate	177-179	0.40	-	2363	1732	1272	7.83 (m, 3H, ArH), 3.84 (s, 3H, OCH_3), 3.35 (m, 2H, CH_2 of C_2H_5,ester), 1.26 (m, 3H, CH_3 ofC_2H_5)	111.09, 119.13, 123.57, 128.97, 151.62, 158.89 (6C, phenyl nucleus), 56.34, 56.45 (2C,OC2H5), 166.65 (C=O, COCH3), 61.21, 14.45 (2C, OC_2H_5)
E3	Ethyl 3,4,5-trihydroxybenzoate	318-320	0.42	3391		1736	1276	8.23 (m, 3H, ArH), 4.71 (s, 1H, OH of phenolic hydroxyl), 3.84 (s, 3H, OCH_3), 3.13 (m, 2H, CH_2 of C_2H_5,ester)	108.15, 116.89, 128.43, 139.34, 148.34, 148.45 (6C, phenyl nucleus), 165.45 (C=O, COC2H5), 59.71, 15.21(2C, OC_2H_5)
E4	Ethyl 4-hydroxy-3,5-dimethoxybenzoate	189-191	0.48	3399		1735	1339	8.73 (m, 3H, ArH), 4.75 (s, 1H, OH of phenolic hydroxyl), 3.29 (s, 3H, OCH_3), 3.00 (m, 2H, CH_2 of C_2H_5,ester)	108.65, 111.25, 125, 142.01, 149.57, 147 (6C, phenyl nucleus), 165.51 (C=O, COC_2H_5), 57.29, 55.43 (2C, OCH3), 63.55, 14.97 (2C,OC_2H_5)
E5	Ethyl 3,4-dihydroxybenzoate	207-209	0.42	3340		1743	1226	7.87 (m, 3H, ArH 3.13), 4.71 (s, 1H, OH of phenolic hydroxyl), 3.85 (m, 2H, CH_2 of C_2H_5,ester)	117.28, 120.31, 124.48, 131.53, 149.32, 153.24 (6C, phenyl nucleus), 166.15 (C=O, COC_2H_5), 61.28, 15.71 (2C, OC_2H_5)
E6	Ethyl 3-(4-hydroxy-3,5-dimethoxyphenyl)acrylate	205-207	0.50	3347	2928	1732	1269	8.23 (m, 3H, ArH), 4.70 (s, 1H, OH), 3.68 (m, 2H, CH_2 of C_2H_5,ester)	109.14, 112.39, 140.85, 142.16, 149.71, 150.12 2 (6C, phenyl nucleus), 169.15 ((C=O, COCH3), 55.61, 57.39 (2C, OC2H5), 62.35, 13.98 (2C, OC_2H_5)
E7	Ethyl 3-(4-hydroxy-3-methoxyphenyl)acrylate	158-160	0.50	340-4	2345	1742	1289	7.38 (s, 2H, OCH_3), 8.49 (m, 3H, ArH), 4.65 (s, 1H, OH of phenolic hydroxyl), 3.85 (s, 3H, OCH_3), 3.45 (m, 2H, CH_2 of C_2H_5,ester), 1.99 (m, 3H, CH_3 ofC_2H_5)	118.19, 121.324, 132.55, 138.16, 147.53. 151.87(6C, phenyl nucleus), 117.91, 149.25 (2C, C=C), 164.56 (C=O, COC2H5), 61.45, 13.98 (2C,OC_2H5)

E8	Ethyl 3-(4-hydroxyphenyl)acrylate	112-114	0.32	3044		1739	1215	7.31 (m, 3H, ArH), 5.78 (s, 1H, OH of phenolic hydroxyl), 3.16 (m, 2H, CH_2 of C_2H_5,ester), 1.27 (m, 3H, CH_3 ofC_2H_5)	117.32, 128.14, 147.26, 133.42, 137.56, 157.89 (6C, phenyl nucleus), 112.52, 151.37 (2C, C=C), 168.56 (C=O, COC2H5), 63.19, 15.01 (2C , OC_2H_5)
E9	Ethyl 2,5-dihydroxybenzoate	185-187	0.40	3421	1745		1247	7.93 (m, 3H, ArH), 3.707 (s, 1H, OH), 3.63(m, 2H, CH_2 of C_2H_5,ester), 1.25 (m, 3H, CH_3 of C_2H_5)	115.52, 118.97, 125.14, 129.20, 152.15, 158.51 (6C, phenyl nucleus), 169.75 (C=O, COC2H5), 61.29, 13.78 (2C , OC_2H_5)
E10	Ethyl 3-(3,4-dihydroxyphenyl)acrylate	224-226	0.32	3440	1739		1241	6.81 (m, 3H, ArH), 5.76 (s,1H, OH of phenolic hydroxyl), 3.36 (m, 2H, CH_2 of C_2H_5,ester),1.38 (m, 3H, CH_3 of C_2H_5)	114.28, 125.85, 132.56, 139.43, 143.77, 148.31 (6C, phenyl nucleus), 120.34, 144.59 (2C, C=C), 169.45(C=O, COCH3), 59.97, 14.24 (2C , OC2H5)
E11	Ethyl 4-methoxybenzoate	129-131	0.48		1741	2345	1221	8.28 (m, 3H, ArH), 3.44 (s, 3H, OCH_3), 2.52 (m, 2H, CH_2 of C2H5),3.63(m, 2H, CH_2 of C_2H_5,ester),1.23 (m, 3H, CH_3 of C_2H_5)	117.72, 121.11, 129.68, 135.42, 138.89, 164.78 (6C, phenyl nucleus), 167.22(C=O, $COC2H_5$), 60.14, 14.55 (2C , OC_2H_5)
P1	Propyl 4-hydroxy-3-methoxybenzoate	152-154	0.48	3422	1732	2345	1458	7.421 (m, 3H, ArH), 4. 601 (s,1H, OH of phenolic hydroxyl), 3.56(s, 3H, -CH_3 of C_3H_7,ester), 1.40 (m, 2H, CH_2 of C_3H_7)	116.41, 119.19, 127.28, 129.10, 147.17, 158.28 (6c, phenyl nucleus), 55.08(1C,OCH_3), 165.26 (C=O, COC_3H_7), 68.12, 20.77, 10.56 (3C, OC_3H_7)
P2	Propyl 3,4-dimethoxybenzoate	187-189	0.40	3422	1749	2362	1450	7.28 (m, 3H, ArH), 4. 91 (s,1H, OH of phenolic hydroxyl), 3.47 (s, 3H, -OCH_3), 1.91(m, 2H, CH_2 of $C_3H_7$3.76 (s, 3H, -CH_3 of C_3H_7,ester)	114.19, 121.54, 129.17, 130.97, 152.21, 159.70 (6C, phenyl nucleus), 55.24, 54.51 (2C,OC3H7), 165.35 (C=O, COCH3), 69.21, 23.15, 10.45 (2C, OC3H7)
P3	Propyl 3,4,5-trihydroxybenzoate	229-231	0.48	3391	1726		1451	8.28 (m, 3H, ArH), 3.67 (s,1H, OH of phenolic hydroxyl), 0.88 (m, 3H, CH_3 of C_3H_7), 1.23(m, 2H, CH_2 of C_3H_7)	107.42, 118.89, 127.13, 141.29, 149.14, 150.31 (6C, phenyl nucleus), 164.42 (C=O, COC_3H_7), 68.24, 19.98, 11.04 (3C, OC_3H_7)
P4	Propyl 4-hydroxy-3,5-dimethoxybenzoate	198-200	0.50	3146	1744		1469	7.32 (m, 3H, ArH), 3.66 (s,1H, OH of phenolic hydroxyl), 0.92 (m, 3H, CH_3 of C_3H_7), 1.42(m, 2H, CH_2 of C_3H_7), 3.55(s,3H, CH_3 of C_3H_7 ester)	111.31, 116.75, 127.21, 144.10, 150.37, 148.14 (6C, phenyl nucleus), 164.38 (C=O, COC3H7), 56.15, 56.31 (2C, OCH3), 68.15, 22.45, 12.35 (3C,OC3H7)
P5	Propyl 4-hydroxy-3,5-	220-222	0.42	3246	1741		1445	7.37 (m, 3H, ArH), 3.69(s,1H, OH of	119.32, 121.25, 125.39, 132.53,

	dimethoxybenzoate							phenolic hydroxyl),1. 42(m, 2H, CH_2 of C_3H_7) 0.92 (m, 3H, CH_3 of C_3H_7), 3.43(s,3H, CH_3 of C_3H_7 ester)	150.24, 155.15 (6C, phenyl nucleus), 165.36 (C=O, COC3H7)
P6	Propyl 3-(4-hydroxy-3,5-dimethoxyphenyl)acrylate	215-217	0.52	3404	1732	2363	1445	7.53 (m, 3H, ArH), 3.51(s,3H, CH_3 of C_3H_7,ester), 1. 23(m, 2H, CH_2 of C_3H_7)	108.41, 116.39, 139.73, 144.58, 151.62, 154.79 (6C, phenyl nucleus), 168.34 (C=O, COC_3H_7), 55.31, 57.38 (2C, OC3H7)
P7	Propyl 3-(4-hydroxy-3-methoxyphenyl)acrylate	169-171	0.46	3403	1730	2364	1430	7.42(m, 3H, ArH), 3.90(s, 1H, OH of phenolic hydroxyl) 3.69(s, 3H,- CH_3 of C_3H_7,ester),1. 21(m, 2H, CH_2 of C_3H_7)	120.50, 122.32, 137.55, 140.80, 149.13. 152.35 (6C, phenyl nucleus), 120. 82, 151.15 (2C, C=C), 163.56 (C=O, COC3H7), 68.25, 19.89, 11.23 (3C, OC3H7)
P8	Propyl 3-(4-hydroxyphenyl)acrylate	124-126	0.36	3400	1742	2364	1230	7.28(m, 3H, ArH), 4.91(s,1H, OH of phenolic hydroxyl) 3.70(s,3H, CH_3 of $C_3H_7$3.51(s,3H, CH_3 of C_3H_7 ,ester),1. 99(m, 3H, CH_3 of C_3H_7)	115.14, 127.65, 146.89, 131.49, 140.55, 160.89 (6C, phenyl nucleus), 116.28, 153.78 (2C, C=C), 165.23 (C=O, COCH3), 69.53, 20.77, 11.12 (3C ,OC3H7)
P9	Propyl 2,5-dihydroxybenzoate	218-220	0.40	3350	1748		1266	7.44(m, 3H, ArH), 4.71(s,1H, OH of phenolic hydroxyl) 3.29(s,3H, -$CH_3$of C_3H_7,ester),2.03(m, 2H, $CH_2$of C_3H_7)	118.34, 120.45, 127.32, 130.42, 154.15, 159.53 (6C, phenyl nucleus), 165.65 (C=O, COCH3), 70.38, 22.56, 11.89 (3C , OC3H7)
P10	Propyl 3-(3,4-dihydroxyphenyl)acrylate	219-221	0.39	3349	1735		1286	8.28 (m, 3H, ArH), 3.47(s,1H, OH of phenolic hydroxyl),1. 25(m, 2H, CH_2 of C_3H_7) 0.88 (m, 3H, CH_3 of C_3H_7)	116.45, 127.97, 134.16, 140.28, 145.25, 150.32 (6C, phenyl nucleus), 122.74, 147.26 (2C, C=C), 167.45(C=O, $COC3H_7$), 69.78, 10.24, 21.87 (3C , OC_3H_7)
P11	Propyl 4-methoxybenzoate	197-199	0.58		1729	2325	1214	7.56 (m, 3H, ArH), 3.21 (s, 3H, OCH_3), 2.72 (m, 3H, CH_2 of C_3H_7), 3.52 (m, 2H, CH_2 of C_3H_7,ester),1.12 (m, 3H, CH_3 of C_3H_7)	119.72, 120.11, 127.68, 132.42, 141.89, 169.78 (6C, phenyl nucleus), 168.21(C=O, COC_3H_7), 61.14, 15.23 (2C , OC_3H_7)

***In-vitro* Antimicrobial Activity:** The synthesized alkali esters showed good antimicrobial activity against the selected bacterial and fungal strains. The MIC values of the test, as well as standard compounds, have been summarised in **Table 3**. The results of antibacterial screening indicated that compound M1 (MIC=6.25 µg/mL), compound E1 (MIC = 6.25µg/mL) containing a hydroxyl group and methoxy group in adjacent position on ring and compound P10 (MIC=6.25µg/mL) containing two hydroxyl group at adjacent position exhibited promising activity against *K. pneumonia*. Compound E3 (MIC=12.5µg/mL) and P10 (MIC=12.5 µg/mL) having a hydroxyl group at ortho position effectively inhibited the growth of P. mirabilis. However, compound P10 (MIC 12.5

µg/mL) having a long alkyl chain was found to inhibit the growth of *E. coli* to a lesser extent than a short alkyl chain containing E3 (MIC = 6.25 µg/mL). Moreover, compound P10 and compound E3 were also found to be better antimicrobials than standard compounds against *S. aureus* (MIC = 25 µg/mL) and against *P. aeruginosa* (MIC = 6.25 µg/mL). The results of antifungal activity indicated that compound P10 exhibited better activity than standard compound against the tested strains and exhibited better activity against *C. albicans* (MIC= 6.25 µg/mL) however, the E3 exhibited the comparable activity to that of the standard antifungal drug (25 µg/mL). The antifungal activity against A. niger for the compound P10 was found to be (MIC= 12.5 µg/mL), compound E3 (MIC= 12.5 µg/mL), while compound E8 (MIC=12.5 µg/mL) with a single electron-donating group was also found potent against *A. niger*. The standard drugs for comparison of antibacterial results were streptomycin, while fluconazole was used as a standard for antifungal evaluation.

TABLE 3 MIC OF SYNTHESIZED ESTERS OF NATURAL ACIDS

Compound(s)	*K. pneumonia*	*E. coli*	*P. mirabilis*	*S. aureus*	*P. aeruginosa*	*C. albicans*	*A. niger*
M1	6.25	25	25	12.5	12.5	25	12.5
M2	25	12.5	25	50	25	50	50
M3	12.5	12.5	25	12.5	12.5	25	25
M4	25	12.5	50	25	12.5	50	12.5
M5	25	25	25	25	25	25	25
M6	12.5	25	12.5	25	25	50	12.5
M 7	25	25	25	50	25	50	25
M8	25	25	50	50	12.5	25	25
M9	12.5	50	25	50	25	50	25
M10	12.5	25	50	25	25	25	12.5
M11	25	25	50	50	12.5	50	12.5
E1	6.25	12.5	25	25	25	50	25
E2	25	12.5	25	50	12.5	25	50
E3	6.25	6.25	12.5	25	6.25	25	12.5
E4	50	25	12.5	50	25	50	50
E5	25	50	25	25	50	50	25
E6	50	25	50	50	25	25	25
E7	25	25	12.5	50	50	25	25
E8	25	25	12.5	50	50	25	6.25
E9	12.5	12.5	25	50	50	25	50
E10	50	25	25	50	25	50	50
E11	25	50	50	50	50	25	25
P1	12.5	12.5	25	25	50	50	25
P2	50	25	25	25	50	25	50
P3	12.5	12.5	25	50	25	50	25
P4	12.5	25	12.5	50	25	25	50
P5	50	25	50	50	50	50	25
P6	25	25	50	50	25	50	25
P7	25	50	50	25	50	25	50
P8	50	25	25	50	50	25	25
P 9	12.5	25	12.5	50	50	50	12.5
P10	6.25	12.5	12.5	25	6.25	6.25	12.5
P11	12.5	25	25	12.5	50	50	12.5
Streptomycin	6.25	50.0	25.0	50.0	6.25	--	--
Fluconazole	--	--	--	--	--	25.0	12.5

Preservative Efficacy: The results of the preservative efficacy study of the selected synthesized compounds in cellulose slurry were performed and summarized in **Table 4**. The log CFU/mL for ethyl 3, 4, 5-trihydroxybenzoate (E3), and propyl 3-(3, 4-dihydroxyphenyl) acrylate (P10) revealed that the values were within the prescribed limit of USP standard criteria. The selected compounds E3 and P10 reduced the growth of microbes on the 14^{th} and 28^{th} day from the initial count, and there is more than 1.0 log reduction from the initial count. The results of preservative efficacy were also found comparable to standard preservatives taken.

TABLE 4 LOG CFU/ML VALUES OF THE SELECTED COMPOUNDS IN CELLULOSE SLURRY

Compound(s) CFU/mL	*E. coli*		*P. aeruginosa*		*S. aureus*		*C. albicans*		*A. niger*	
	14 d	28 d	14 d	28 d	14 d	28 d	14 d	28 d	14 d	28 d
E3	2.14	2.11	2.12	2.06	2.27	2.20	2.00	1.96	1.99	1.12
P10	1.83	1.70	2.06	2.02	2.90	2.20	2.23	2.03	2.39	2.20
Sodium Benzoate	2.20	2.03	2.30	2.20	2.90	2.53	2.50	2.20	2.30	2.01
Methyl Paraben	2.33	2.25	2.33	2.03	2.20	2.10	2.03	1.90	2.20	2.03
Ethyl Paraben	2.36	2.00	2.36	2.00	2.90	2.20	2.96	2.53	2.44	2.02

*Initial microbial count present in inoculums 1x105-1x106 CFU/mL

Anti-oxidant Activity: Antioxidant potential of all the synthesized esters were evaluated by DPPH radical scavenging assay method, and result were summarized in **Table 5.** Further, the results revealed that the compound E3 (IC_{50} value 6.48±0.001µM) and P10 (IC_{50} value 06.09 ± 0.042µM) were found more potent antioxidants than reference l-ascorbic acid (IC_{50} value 8.5.18±0.009 µM). Compound M3 and P7 also showed comparable antioxidant potential to reference with IC_{50} values as 08.39 ± 0.007 µM and 08.22 ± 0.012 µM, respectively. Both had hydroxyl groups at meta position where hydroxyl groups act as electron-withdrawing, thus facilitating hydrogen release from acid derivatives. While compound M5 (IC_{50} value 18.80 ± 0.003 µM) and E5 (IC_{50} value 13.73 ± 0.045µM) exhibited the lowest antioxidant activity because of the presence of hydroxyl group at adjacent positions on the phenolic ring, the adjacent arrangement leads to stabilization of molecule against the release of hydrogen ion.

TABLE 5: DPPH RADICAL SCAVENGING ACTIVITIES OF SYNTHESIZED DERIVATIVES

Compound(s)	IC_{50} (µM)[a]	Compound(s)	IC_{50} (µM)[a]
M1	09.19 ± 0.001	E1	10.94 ± 0.025
M2	11.94 ± 0.025	E2	11.95 ± 0.031
M3	8.39 ± 0.007	E3	06.48 ± 0.001
M4	11.22 ± 0.012	E4	10.49 ± 0.028
M5	18.80 ± 0.003	E5	13.73 ± 0.045
M6	15.37 ± 0.054	E6	10.80 ± 0.054
M7	8.5.18 ± 0.009	E7	10.11.18 ± 0.032
M8	12.95 ± 0.031	E8	09.21 ± 0.001
M9	11.94 ± 0.025	E9	11.12 ± 0.032
M10	08.09 ± 0.042	E10	08.09 ± 0.042
M11	11.22 ± 0.012	E11	11.22 ± 0.012
P1	11.83 ± 0.004	P7	08.22 ± 0.012
P2	11.94 ± 0.025	P8	12.95 ± 0.031
P3	07.93 ± 0.013	P9	13.22 ± 0.021
P4	11.47 ± 0.043	P10	06.09 ± 0.042
P5	19.19 ± 0.001	P11	11.5 ± 0.009
P6	15.37 ± 0.054	Ascorbic acid	8.5 ± 0.009

[a]Value are expressed as mean ± SEM, n = 3

CONCLUSION: This study has ascertained that the derivatives of phenolic acids possessed the excellent preservative ability. Based on the antimicrobial results of the present study, methyl 4-hydroxy-3-methoxybenzoate and ethyl 4-hydroxy-3-methoxybenzoate have demonstrated better antimicrobial activity that is comparable to standard compounds, and both the compounds showed better antioxidant potential than standard l-Ascorbic acid. Further, the preservative efficacy test clearly showed that ethyl 3, 4, 5-trihydroxy-benzoateand propyl 3-(3, 4-dihydroxyphenyl) acrylate were effective against all selected strains used during the study and even better than reference compounds in the case of *E. coli* and *S. aureus*.

Data Availability: The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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