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Comparative evaluation of the antioxidant capacity of ferulic acid and synthesized propionyl ferulate

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ABSTRACT

Ferulic acid (FA) is a polyphenolic compound with demonstrated antioxidant capacity. In this study, propionyl ferulate (PF) was synthesized and characterized using melting point, ultraviolet spectroscopy, Fourier-transform infrared spectroscopy, and mass spectrometry. The propionyl ferulate was comparatively evaluated for antioxidant potential which included the ability to quench reactive species of 2,2-diphenyl-2-picrazyl-hydrazyl (DPPH), hydroxyl, nitric oxide, and superoxide anion. In addition, the total antioxidant capacity and membrane stabilizing properties of the ferulate were determined. Comparatively, the spectroscopically characterized PF showed superior scavenging capacity for the DPPH, hydroxyl, and nitric oxide free radicals when compared to FA. On the contrary, PF showed a poor scavenging capacity for superoxide anion radicals. Furthermore, PF showed little or no potential for membrane stability. In conclusion, the data suggest that structurally modifying FA to PF improved the antioxidant capacity for several free radicals.

INTRODUCTION

Antioxidants are chemical or molecular substances that delay or arising from the action of reactive oxygen or nitrogen species (ROS/RNS). They function by electron donation, metal ion chelation, antioxidation, or gene expression regulation. Antioxidants have displayed therapeutic potential in several ailments, such as neurodegenerative diseases, cardiovascular and cancer (Kim *et al*., 2003; Soobrattee *et al*., 2005) resulting in rapidly increasing interest in their medicinal application (Wang and Lin, 2000). In addition, the demand for antioxidants in various manufacturing processes, particularly the food industry, has been on the increase, leading to the production of synthetic antioxidants to match this demand. However, in recent years, there has been a shift from synthetic to natural antioxidants (Adeyemi *et al*., 2018). The preference for the use of natural antioxidants stems from the belief that they have lower toxicity than their synthetic counterparts.

Polyphenolic compounds are naturally occurring antioxidants that are ubiquitous in plant and vegetable diets (Clifford, 1999; Pimentel *et al*., 2005). This class of natural compounds has also received interest within the past decade as a result of their therapeutic prospects, particularly against cardiovascular-related diseases and cancer (Nardini, 2004). Ferulic acid (4-hydroxy-3-methoxycinnamic acid) (FA), is a natural phenolic and it is one of the most abundant phenols in many plants (D'Archivio *et al*., 2007; Rechner *et al*., 2001). FA occurs freely, or in dimers, or as esters bound to polysaccharides or proteins in cell walls, such as arabinoxylan in grass and xyloglucan in bamboo (Fazary and Ju, 2007; Rumbold *et al*., 2003). FA consists of a hydroxyl, carboxyl, and methoxy group, an ethylenic bond, and a benzene ring (Zhang *et al*., 2003). The chemical features of FA have been exploited in the synthesis of diverse derivatives such as esters, ethers, amides, anhydrides, acyl chlorides, acid polymers,

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nitrobenzenes, benzene sulfonic acids, and benzene halides of FA (Zhang *et al*., 2003). The scavenging properties of FA may be attributed in presence of electron-donating groups; 3-methoxy and 4—hydroxyl (Kanasaki *et al*., 2002). One of these characteristics is the presence of the electron-donating groups on the ring structure (3-methoxy and 4-hydroxyl). The second is the presence of a carboxylic acid group adjacent to an unsaturated carbon-carbon double bond (Kanasaki *et al*., 2002). The carboxylic acid group also provides support, through which it binds to membrane bilayers, thereby preventing lipid peroxidation (Kanasaki *et al*., 2002). Furthermore, FA has the capacity to negate radical chain reactions through polymerization and is known to increase cross-linkages in polysaccharides and other polymers, giving it capacity to prevent UV-radiation damage (dos Santos *et al*., 2008).

Although FA has been shown to have a low toxicity index relative to its various biological and medicinal activities, its clinical use is still underexplored. Its use is greatly limited by the fact that it is unstable in different solvents and has a low interaction with lipids (Compton *et al*., 2012; Qin *et al*., 2013; Stamatis *et al*., 2001). Therefore, the focus has shifted to the use of FA derivatives as possible alternatives to FA due to their lipophilic properties. In this study, we synthesized propionyl ferulate (PF) and evaluated the antioxidant potential of the derivative compared with FA.

MATERIALS AND METHODS

Experimental

Analytical grade reagents used included FA and L-ascorbic acid (Sigma-Aldrich, St. Louis, MO). The monitoring of reaction and purity check were accomplished by thin-layer chromatography (TLC) on precoated silica gel (0.25 mm 60 F_{254} plates, Merck, Germany) and observed in UV light (254 and 365 nm). A stock solution (2 mg/ml) of PF or FA was prepared in methanol.

Synthesis of PF

First, the reaction was initiated by dissolving 0.97 g of FA (5 mmol, 194 g) in 5% NaOH (aq). After 30 min of continuous stirring on ice (10°C) the resulting solid was collected via filtration and washed with cold saturated brine solution to purify (Scheme 1). The product obtained was dried at room temperature.

Propionyl ferulate

It is a white powder, having m.p. 162-164°C, yield 37%, (R_f is 0.60 in dichloromethane), (λ_{max} is 321 nm with 2.5 abs), IR using the KBr pellet method; V_{max}, cm^{-1} - 3437, 2939, 2366, 1687, 1625, 1425, 1126; $C_{13}H_{14}O_5$; MW=250.25; MS: *m/z* (%): 150, 135, 118, 107.

The melting point was obtained by using a melting point apparatus (Electrothermal, UK), whereas absorption data were recorded by using a UV-VIS spectrophotometer (Beckman Coulter DU 730 Life Sciences, UK). The infrared spectrum was obtained by the KBr pellet protocol on a Shimadzu (8400S) Fourier-transform infrared spectrometer. The molecular weight was estimated by mass spectrometry in ionization mode at 70 eV (MS-QP 2010 PLUS, Shimadzu, Japan), with Finnigan MAT ion trap detector.

In vitro antioxidant screening

Assay for 2,2-diphenyl-2-picryl-hydrazyl (DPPH) scavenging potential

The DPPH radical scavenging assay was performed in line with the method described by Devi *et al*. (2011). An aliquot (0.1 ml) of PF, FA, or L-ascorbic acid (concentration range: 0.01, 0.05, and 0.1 µg/ml, respectively) was added to 2.9 ml of 0.1 mM DPPH-methanol. After 30 min of vigorous shaking and incubation in the dark, the absorbance of mixture was read at 517 nm on a spectrophotometer (Jenway, Staffordshire, UK). The control contained 0.1 ml of methanol and 2.9 ml of DPPH, whereas methanol only was used as blank. The % activity was estimated using the following expression:

$$\% \text{ Activity} = \left[1 \left(\frac{\text{Absorbance of sample} - \text{Absorbance of blank}}{\text{Absorbance of control}} \right) \right] \times 100$$

Assay for nitric oxide radical scavenging potential

The nitric oxide radical scavenging assay was carried out as described by Ilavarasan *et al*.. (2005). An aliquot (0.5 ml) of PF, FA, or L-ascorbic acid (concentration range: 0.01, 0.05, and 0.1 µg/ml, respectively) was added to 0.5 ml of sodium nitroprusside. This mixture was incubated for 5 hours at 25°C. Thereafter, 0.5 mL of the incubated mixture was added to 0.5 mL of Greiss reagent, and the absorbance was read at 540 nm on UV/VIS spectrophotometer. The blank contained 0.5 ml of distilled water, 0.5 ml of sodium nitroprusside, and 0.5 ml of Griess reagent and had an absorbance of 540 nm. The following expression was used to estimate the % activity:

$$\% \text{ Activity} = \frac{\{(\text{Abs control} - \text{Abs sample})\}}{(\text{Abs control})} \times 100$$

Assay for superoxide anion scavenging potential

This assay was carried out using as described elsewhere (Luo *et al*., 2002). Briefly, 1 mL of PF, FA, or L-ascorbic acid (concentration range: 0.01, 0.05, and 0.1 µg/mL) was added to 4.5 mL of Tris-HCl buffer. After 20 min incubation at 25˚C, 0.4 mL of pyrogallol was added and reaction allowed for 4 min. This was followed by termination of reaction with 0.1 mL of HCl. The mixture was pelleted at 1,000 g for 15 minutes (model C5, LW Scientific, GA), and the absorbance at 325 nm was recorded on UV/VIS spectrophotometer.

The blank contained 1ml of distilled water, 4.5 ml of Tris-HCl buffer, 0.4 ml of pyrogallol, and 0.1 ml of HCl. The % activity was estimated using the following expression:

$$\% \text{ Activity} = \frac{\{(\text{Abs control} - \text{Abs sample})\}}{(\text{Abs control})} \times 100$$

Assay for total antioxidant capacity

The assay was performed as described previously (Saha *et al*., 2019). Briefly, 0.3 ml of PF, FA, or L-ascorbic acid

(concentration range: 0.01, 0.05, and 0.1 µg/ml, respectively) was added to 3 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). This mixture was incubated at 95°C for 90 minutes. This was followed by cooling mixture at room temperature and recording the absorbance at 695 nm on a spectrophotometer against a blank. For control, methanol was used instead of the PF or FA and the activity was expressed as gram equivalents of L-ascorbic acid.

Assay for membrane stabilizing effect

This assay was done following a method described by Malomo *et al*. (2011). The assay mixture consisted of 2 ml of hyposaline, 1 ml of phosphate buffer, erythrocyte suspension in 1.5 ml of isosaline, and 1 ml of PF, FA, or reference compound (concentration range: 0.01, 0.05 and 0.1 µg/ml, respectively). The control lacked the drug or test samples, whereas the drug control and test samples lacked the erythrocyte suspension. Isosaline was added to the controls to make the reaction volumes up to 4.5 ml. After 30 min of incubation at 56˚C, the mixture was cooled and pelleted at 1000 g for 15 min.

The absorbance of the supernatant was read at 560 nm on UV/VIS spectrophotometer. The % membrane stabilizing activity was estimated as follows:

$$\% \text{ Activity} = \left(\frac{\text{Drug or Sample test value} - \text{Drug or Sample Test control}}{\text{Control Value}} \right) \times 100$$

Statistical analysis

Results were analyzed by a one-way ANOVA (GraphPad Prism 5, CA, USA) and are presented as the mean of replicates ± standard error of mean (SEM). The Tukey's post-Hoc was used for multiple comparisons and level of significance was taken at $p < .05$.

RESULTS

Synthesis and characterization of PF

The synthesis of PF was accomplished by treating FA dissolved in sodium hydroxide with propionic anhydride. The intermediate sodium salt of FA facilitated the reaction with propionic anhydride. The reaction was monitored by TLC and reached completion in 30 minutes.

The infrared spectrum of the product obtained (PF), a white powder with a melting point of 162°C–164°C, showed strong absorption bands at 3,437 and 1,687 cm^{-1} corresponding to OH and CO stretching vibrations, respectively Supplementary data.

In vitro antioxidant assays

The comparative evaluation for the *in vitro* antioxidant potential showed that PF had better scavenging properties against DPPH, hydroxyl, and nitric oxide radicals compared with FA (Figs. 1–3). At all concentrations, PF showed superior scavenging activity against DPPH compared with FA or L-ascorbic acid. Similarly, PF at 0.01 and 0.05 µg/ml exhibited better scavenging activity against the hydroxyl radical compared with FA or L-ascorbic acid. In addition, PF showed a strong nitric oxide radical scavenging activity compared with FA or L-ascorbic acid. On the contrary, FA showed a greater scavenging activity against the superoxide anion (Fig. 4). FA also had a higher total antioxidant and membrane-stabilizing capacity than PF (Figs. 5 and 6). Meanwhile, acetylsalicylic acid which was positive drug control for the membrane stability assay showed better membrane stability than either FA or PF. Indeed, PF showed the least membrane-stabilizing capacity across all concentrations.

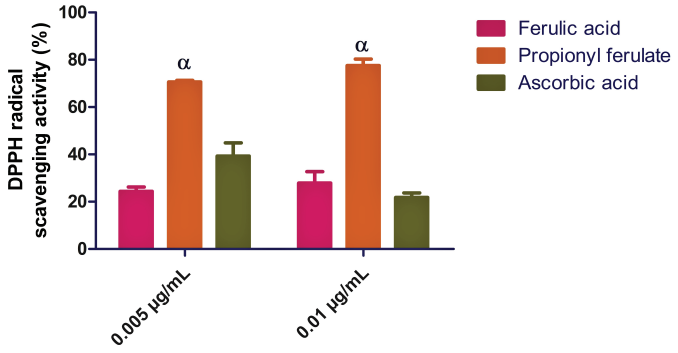


Figure 1. DPPH radical scavenging activity of propionyl ferulate, ferulic acid, and L-ascorbic acid (standard). Data are expressed as the mean of three replicates ± SEM. α is significant at $p < 0.05$ versus ferulic acid and/or L-ascorbic acid.

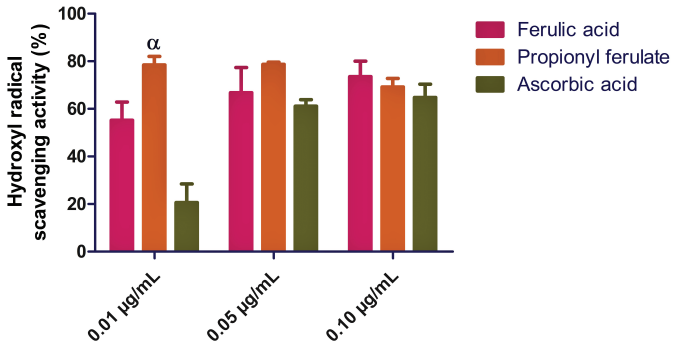


Figure 2. Hydroxyl radical scavenging activity of propionyl ferulate, ferulic acid, and L-ascorbic acid (standard). Data are expressed as the mean of three replicates ± SEM. α is significant at $p < 0.05$ versus ferulic acid and/or L-ascorbic acid.

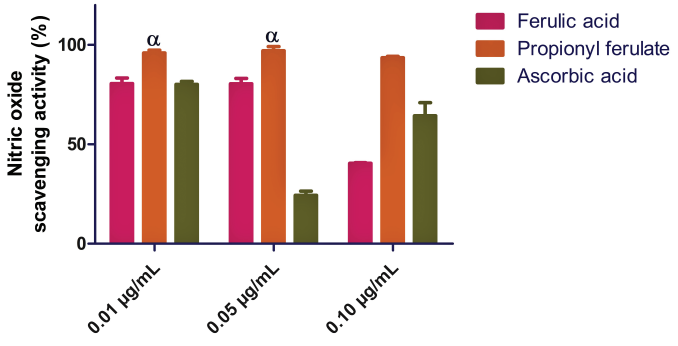


Figure 3. Nitric oxide radical scavenging activity of propionyl ferulate, ferulic acid, and L-ascorbic acid (standard). Data are expressed as the mean of three replicates ± SEM. α is significant at $p < 0.05$ versus ferulic acid and/or L-ascorbic acid.

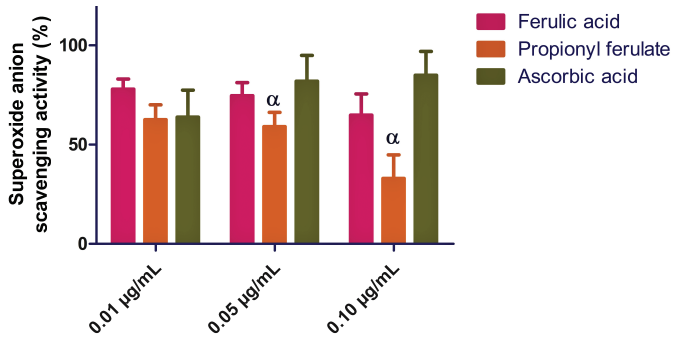


Figure 4. Superoxide anion scavenging activity of propionyl ferulate, ferulic acid, and L-ascorbic acid (standard). Data are expressed as the mean of three replicates ± SEM. α is significant at $p < 0.05$ versus ferulic acid and/or L-ascorbic acid.

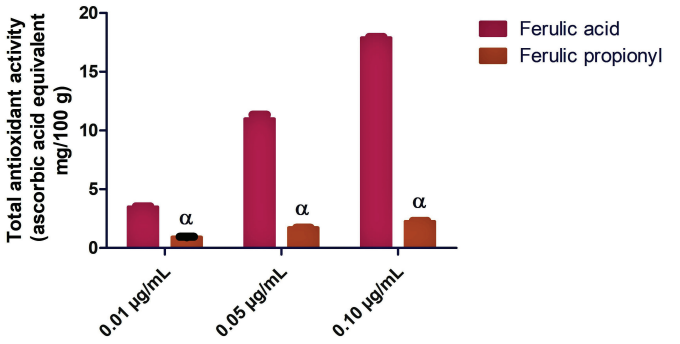


Figure 5. Total antioxidant capacity of propionyl ferulate and ferulic acid expressed as a number of gram equivalents of L-ascorbic acid. Data are expressed as the mean of three replicates ± SEM. α is significant at $p < 0.05$ versus ferulic acid.

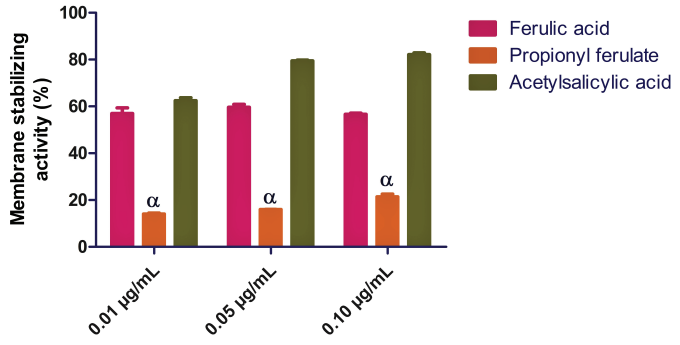
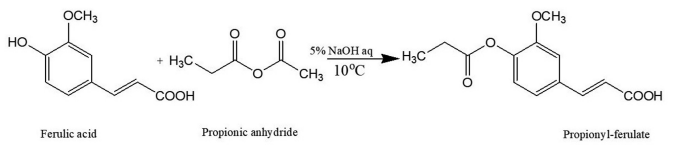


Figure 6. Percentage membrane stabilizing activity of ferulic acid, propionyl ferulate, and acetylsalicylic acid (standard). Data are expressed as the mean of three replicates ± SEM. α is significant at $p < 0.05$ versus ferulic acid and/or acetylsalicylic acid.



Scheme 1. Synthesis of PF.

DISCUSSION

Various methods have been used to synthesize the esters of phenolics to improve their bioactivity (Hosoda *et al*., 2002; Zhao *et al*., 2015). The strong hydrophobicity of FA, a hydroxycinnamic acid, has necessitated the synthesis of derivatives with improved bioactivity and reduced toxicity (Anselmi *et al*., 2005; Nyaradzo *et al*., 2009; Rakotondramanana *et al*., 2007). The synthesis of the PF was accomplished following a standard procedure (Adeyemi *et al*., 2019). The PF synthesized and characterized in this study had UV, IR, and MS data that conform to the indicated structure.

To maximize the antioxidant and/or medicinal value of FA and to circumvent its physicochemical and biological disadvantages, researchers previously synthesized several derivatives (Adeyemi *et al*., 2018; 2019). In this study, we synthesized PF and comparatively evaluated it for antioxidant potential. The data indicate that PF showed a superior antioxidant capacity to either FA or ascorbic acid. It also had a superior scavenging activity for the DPPH radical compared to FA or ascorbic acid. This finding differs from the previous study (Karamac *et al*., 2005) which showed that FA had a better antioxidant capacity than its derivatives. In addition, PF showed better scavenging capacity for hydroxyl and nitrogen radicals when compared with FA or ascorbic acid. These findings combine to indicate that structurally modifying FA to form PF did not compromise its antioxidant potential. This is consistent with the previous findings (Adeyemi *et al*., 2018), in which FA derivatives showed superior antioxidant activity compared with FA.

The superior total antioxidant and membrane-stabilizing capacity observed with FA over PF might be attributable to the polarity of the assay method; thus, since PF is less polar, its functional properties may be inaccessible in the assay. Further experiments are needed to confirm this speculation. PF's poor capacity for membrane stabilization could also be due to its lower hydrophobicity than FA. In this scenario, the reduced hydrophobicity of PF might decrease its interaction with the membrane in the lipid phase, thereby limiting its capacity to offer protection in this phase. This line of thought is further reinforced by the finding that FA showed considerable membrane protection. Meanwhile, FA had better total antioxidant capacity as well as higher scavenging activity for superoxide anion radicals compared with PF. This may not be unexpected since FA possesses strong antioxidant potential (Kanski *et al*., 2002).

FA, which is a monohydrated cinnamic acid, can form derivatives with distinct structural orientations. Thus, evaluating its derivatives is essential to discover the changes in biological activity (Calheiros *et al*., 2009). Of the various approaches for synthesizing compounds from FA, esterification is increasingly popular among scientists because the resulting compounds tend to have the higher antioxidant ability and increased lipophilic properties than FA itself (Adeyemi *et al*., 2018). The alkyl ferulates are esterified ferulates that have been reported to have higher antioxidant activity in a membranous system (Anselmi *et al*., 2005), to prevent neurodegenerative disorders (Anselmi *et al*., 2004), to possess anticancer potential (Sultana, 2012), and to possess the ability to penetrate the skin using intercellular pathways (Murakami *et al*., 2000). Alkyl ferulates include ferulic propyl, which could be single-bonded (-anyl), double-bonded (-enyl), or

triple-bonded (-ynyl). In this study, the focus was on the single-bond derivative, PF. The study showed that this structural modification of FA did not inhibit the antioxidant property of PF compared with FA but, rather, enhanced it. This supports the idea that the degree of freedom introduced by the FA modification, especially by esterification, may be indicative of rotation around the (C-O) bond, which could be a destabilizing factor that could increase their scavenging property (Calheiros *et al*., 2009; Zhang *et al*., 2010).

CONCLUSION

Findings revealed that PF had higher antioxidant activities than FA. The data indicate that structurally modifying FA can significantly improve its antioxidant activity. Additional investigations are warranted to explore the bioactive prospects of PF.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHOR CONTRIBUTIONS

OSA—concept, analysis, drafting, and final review; ADA—data collection, analysis, and final review; OJA—data collection, analysis, final review; OA—concept, analysis, drafting, final review; OA—data collection analysis and final review; AI—data collection, analysis, and final review; DR—data collection, analysis, and final review; TCE—data collection, analysis, and final review; GEB—data collection, analysis, and final review; JOO—data collection, analysis, and final review; OOO—data collection, analysis, and final review.

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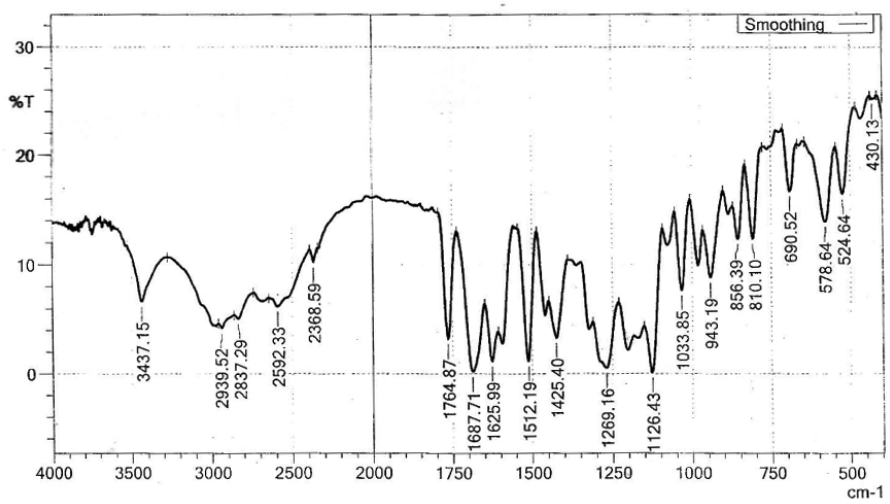
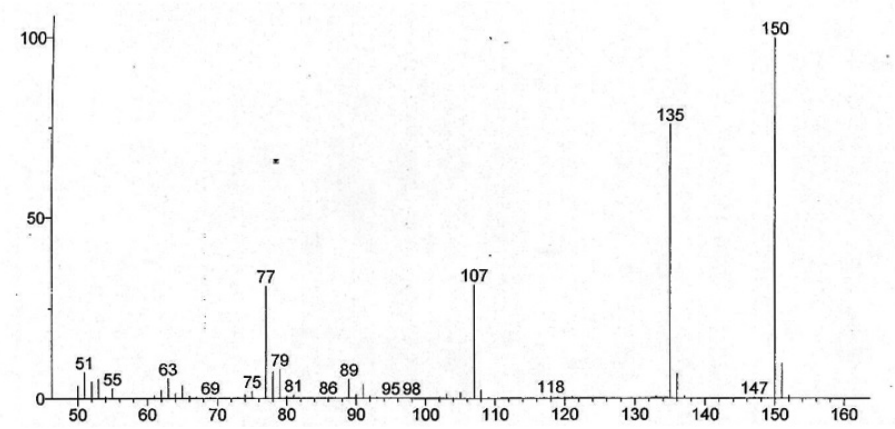
SUPPLEMENTARY DATA



FT-IR Spectrum of Product



Mass Spectrum of Product