Phenolic compounds are widely distributed in the plant kingdom and in the microorganisms. Cinnamic acid and its hydroxylated derivative—ferulic acid, are phenolic compounds. Ferulic acid possesses antioxidant potential, as well as anti-cancer, anti-inflammatory, and antimicrobial properties. It prevents the harmful effects of radiation both as an ultraviolet absorber and as a free radical scavenger; it is not cytotoxic. Although ferulic acid has beneficial properties, it is hardly used in cosmetic preparations and has been rarely studied in the literature. Herein, we review the literature on ferulic acid, to provide information which can contribute to further research on the compound.

KEYWORDS: Ferulic acid. Properties. Antioxidant activity. Antimicrobial activity. Cytotoxicity. Analytical methods.

INTRODUCTION

Phenolic compounds are a group of antioxidants that act against free radicals and consequently prevent cell aging. Their molecules are characterized by a benzene ring, a carboxylic group, and one or more methoxyl and/or hydroxyl groups (Soares, 2002). Phenolic compounds are widely distributed in the plant kingdom and in the microorganisms (Simões et al., 2010).

Phenolic compounds include phenolic acids. The occurrence of these compounds in the plant kingdom allows them to be classified into two groups: widely distributed phenolic compounds and phenolic compounds with restricted distribution. The first group includes derivatives of benzoic and cinnamic acids, coumarins, flavonoids, and polymerization derivatives (tannins and lignins). The second group consists of other compounds (Simões et al., 2010).

Phenolic acids can further be classified into benzoic acid and cinnamic acid derivatives. Benzoic acid and its derivatives (Figure 1) have seven carbon atoms, and are the simplest in nature; examples are salicylic acid, gentisic acid, p-hydroxybenzoic acid, protocatechuic acid, vanillic acid, gallic acid, and syringic acid (Soares, 2002). Cinnamic acid and its derivatives (Figure 2) have nine carbon atoms. Seven derivatives of cinnamic acid are the most frequently found in the plant kingdom; they include o-coumaric acid, m-coumaric acid, p-coumaric acid, caffeic acid, ferulic acid (FA), and sinapic acid. These derivatives can exist in two isomeric forms because of a double bond in their structure; however, the ones most frequently found in nature have a trans conformation and are more stable (Escarpa and Gonzalez, 2001; Simões et al., 2010).

Among the derivatives of cinnamic acid, FA stands out (Figure 3) (Barberousse, 2008). FA is 3-(4-hydroxy-3-methoxyphenyl)prop-2-enolic acid, its other names include 4-Hydroxy-3-methoxycinnamic acid, caffeic...
acid 3-methyl ether, and coniferic acid (Chowdhury et al., 2016).

FA is present at relatively high concentrations in many plants; in addition, it is bound to cell wall polymers in Poaceae and other forage plant species, within the monocotyledons and the dicotyledons (Barberousse, 2008; Mathew and Abraham, 2004). It is a potent phytochemical and can be obtained from various plant species such as rice, wheat, barley, apple, orange, coffee, and peanut (Chowdhury et al., 2016). FA was first isolated by Hlasiwetz and Barth in 1866 and was first synthesized in 1925 (Graf, 1992).

FA has beneficial properties; however, it is hardly ever used in cosmetic preparations available in the market, and has been rarely studied in the literature. Thus, in this article, we review the literature on FA, to provide information that can contribute to further research on the compound.

FIGURE 1 - Benzoic acids chemical structure.

FIGURE 2 - Chemical structure of the main cinnamic acids.

FIGURE 3 - Ferulic acid.

### PROPERTIES OF FA

FA could exhibit anticancer, anti-inflammatory, and antimicrobial properties. It prevents the harmful effects of radiation as a photo-protective and an antioxidant agent in cosmetic and biomedical preparations (Chowdhury et al., 2016; Ouimet et al., 2013). FA scavenges free radicals even when present in small concentrations (Broinizi, 2007). Its efficacy is reduced by the decarboxylation mechanism, which promotes its decomposition by heat, air, or light (Ouimet et al., 2013). The characteristics of FA are presented in Table I; these characteristics have been obtained using ACD/ChemSketch software and PubChem, and from a previous report (Mota et al., 2008).

| Synonym | 4-Hydroxy-3-methoxy cinnamic acid |
|---------|----------------------------------|
| IUPAC name | 4-Hydroxy-3-methoxy cinnamic acid |
| CAS number | 1135-24-6 |
| Empirical formula | C_{10}H_{10}O_{4} |
| Molecular weight | 194.18 g·mol^{-1} |
| Composition | C (61.85%), H (5.19%), O (32.96%) |
| Density | 1.316 ± 0.06 g·cm^{-3} |
| Melting point | 168–171 °C |
| pKa | 4.61 |
| Log | 1.64+/−0.36 |

TABLE 1 - Characteristics of FA (Data from: ACD/ChemSketch; PubChem; MOTA et al., 2008)

In murine models, FA is intactly absorbed, exhibits the necessary pharmacokinetic properties, and is
Antioxidant activity

Many phenolic compounds exhibit antioxidant activity, whereby they inhibit or reduce the effects of free radicals and oxidizing compounds. Antioxidant activity is an effect of the donation of the hydrogen atom of a hydroxyl (OH) group in their aromatic structure to a free radical (Giada and Mancini, 2010; Magnani et al., 2014; Soares, 2005). The antioxidant potential of FA can be ascribed to its structural characteristics, as shown in Figure 4. Its potent antioxidant activity is explained by the formation of a resonance-stabilized phenoxy radical, because of its phenolic nucleus and an unsaturated side chain. The resonance stabilization accounts for the effective antioxidant potential of FA (Chowdhury et al., 2016; Srinivasan, 2007).

The stability of the phenoxy radical and the increase in the antioxidant efficiency of FA are a result of an OH group in the ortho position with a methoxyl group (electron donor) (Cuvelier et al., 1992; Degáspari and Waszczynskyj, 2004). FA exhibits antioxidant effect against lipid peroxidation, through the effective scavenging of free radicals, which is attributable to its phenolic OH group (Giada and Mancini, 2010). Nazaré (2013) reported that FA has higher antioxidant potential than its ester derivatives.

FA is a strong ultraviolet (UV) absorber owing to the high degree of conjugated unsaturation. Its radiation absorption involves phenoxy radical formation leading to cis-trans isomerization. At high concentrations, FA can also protect other light-sensitive compounds against oxidative damage because it attenuates the amount of UV radiation impinging on the dissolved molecules (Chowdhury et al., 2016).

As reported by Brand-Williams and coworkers (1995), FA reduced one molecule of 2,2-diphenyl-1-picrylhydrazyl, whereas, other phenolic compounds such as coumaric acid, vanillin, and vanillic acid did not. FA has the ability to increase the resistance of low-density lipoprotein to oxidation mediated by metmyoglobin, through the scavenging of peroxyl radicals (Bourne et al., 2007; Castelluccio et al., 1997). The antioxidant activity of FA is higher than that of its derivatives (Kikuzaki et al., 2002).

At very high concentrations, FA disrupted the peroxidation of bovine brain phospholipid liposomes. It also reacts with hypochlorous acid at a rate fast enough to protect α-antiproteinase (Scoot et al., 1993).

In a qualitative comparative study of the kinetic behavior of oxidation inhibition in benzoic acid and cinnamic acid, FA increased the period of oxidation induction twice as much in relation to the control (Marinova and Yanishlieva, 1992; Ramalho and Jorge, 2006). This has also been verified in other kinetic studies, using triacylglycerols and methyl esters of sunflower oil, wherein phenolic acids participated more effectively in the initiation phase of the oxidation and FA, caffeic acid, and sinapic acid also participated in the propagation reactions (Ramalho and Jorge, 2006; Yanishlieva and Marinova, 1995).

FA is an active ingredient in sunscreens and skin lotions indicated for photoprotection, based on its antioxidant property. Its high UV absorbance and radiation-initiated antioxidant potential would afford excellent photoprotection to UV-sensitive biological materials (Graf, 1992).

![Image](image_url)

**FIGURE 4** - FA radical’s Resonance Stabilization.
Antimicrobial activity

The indiscriminate and abusive use of antibiotics has resulted in many pathogenic microorganisms being resistant to antibiotics. Therefore, researchers around the world have been looking for alternatives to minimize or also substitute the classic antimicrobial agents. In addition to the many known properties of phenolic compounds, they have great potential for use as antibiotics. Herein, we will show concrete evidence for use of FA as an antimicrobial agent.

FA is a component of propolis (24 mg.mL−1 of the extract). The composition of propolis was determined by high-performance liquid chromatography (HPLC) and some phenolic compounds such as vanillin, coumaric acid, and FA were identified. The antimicrobial activity of propolis oil extract was evaluated in vitro, and the minimal concentrations of phenolic compounds that inhibited the microorganisms were determined. The results showed that the phenolic compounds in propolis oil extract have antimicrobial property (Ramanauškiene and Inkénienė, 2011).

Methanolic extracts from *Ligusticum mutellina* were obtained and analyzed by HPLC. Chlorogenic acid was the predominant phenolic acid found. In addition, gallic, p-OH-benzoic, caffeic, and p-coumaric acids and FA were identified. The extracts showed high antioxidant activity and moderate antibacterial and antifungal activity (Minimum inhibitory concentration (MIC) = 1.25–2.5 mg.mL−1). *Micrococcus luteus*, *Pseudomonas aeruginosa*, and *Candida spp.* were the most sensitive to the extracts (Sieniawska et al., 2012).

The activity of FA at 1000 μg.mL−1, was evaluated in terms of the prevention and control of biofilms formed by *Pseudomonas aeruginosa*, *Escherichia coli*, *Listeria monocytogenes*, and *Staphylococcus aureus*. In addition, the effects of FA in terms of planktonic cell susceptibility, and on bacterial adhesion and motility were tested. The minimum bactericidal concentration for *P. aeruginosa* was 500 μg.mL−1, while those for *E. coli*, *L. monocytogenes*, and *S. aureus* were 2500 μg.mL−1, > 5000 μg.mL−1, and 5000 μg.mL−1, respectively. FA caused total inhibition of swimming (*L. monocytogenes*) and swarming (*L. monocytogenes* and *E. coli*) motilities. The colony spreading of *S. aureus* was completely inhibited by FA. Furthermore, FA promoted reductions in biofilm activity by > 70% for all the biofilms tested (Borges et al., 2012).

Borges and coworkers (2013) demonstrated that FA showed activity against *S. aureus*, *E. coli*, *L. monocytogenes*, and *P. aeruginosa*, with MIC of 100–1250 μg.mL−1. They also found that FA irreversibly changed the charge, permeability, and physicochemical properties of membranes. Furthermore, pore formation and rupture in the bacterial membranes were observed.

The derivatives of FA were synthetized, and the compounds were evaluated for their antimicrobial activity against different gram-negative and gram-positive bacterial and fungal strains. The results of the antimicrobial screening indicate that all the synthesized compounds presented antimicrobial activity, with variable intensity (Khatak, 2015).

In the study of Yakub and coworkers (2018), FA, in the amorphous state, was embedded in poly (ε-caprolactone) (PCL) fibers or in Ch coating. The release of FA depended on the design and opus of the fibrous materials. The pathogenic bacteria—*S. aureus*, were killed by the non-coated and Ch-coated PCL fibrous mats loaded with FA.

In another study, the effects of FA were tested on *Cronobacter sakazakii*, an opportunistic pathogen transmitted through food and that causes infection mainly in newborns, infants, and immunocompromised adults. FA caused cell membrane dysfunction and changes in cellular morphology. The MIC against *C. sakazakii* strains was 2.5–5.0 mg.mL−1 (Shi et al., 2016).

FA is a component of sweetop fruit, at a concentration of 0.02%. The antimicrobial activity of phenolic compounds extracted from three tropical fruits (persimmon, guava, and sweetop) was tested against 12 targeted pathogens including eight standard strains (*Bacillus cereus*, *S. aureus*, *Staphylococcus epidermidis*, *E. coli*, *Monilia albican*, *Shigella flexneri*, *Salmonella typhimurium*, and *P. aeruginosa*) and four multidrug-resistant strains (methicillin-resistant *S. aureus*, carbapenem-resistant *P. aeruginosa*, extended spectrum β-lactamase-producing *E. coli*, and multidrug-resistant *Acinetobacter baumannii*), which are common in clinical settings. The results were promising because the extract of the three tropical fruits showed high bactericidal activity against the strains tested in the experiment (Fu et al., 2016).

Cota-Arriola and coworkers (2016) prepared chitosan matrices with FA and analyzed the relationship between the addition of FA on the growth of *Aspergillus parasiticus*, a highly toxigenic fungal species for both humans and livestock, commonly found in food
commodities such as cereals. The results demonstrated that the incorporation of FA into the chitosan matrices significantly enhanced its fungistatic activity on growth, spore germination, and morphology of *A. parasiticus*.

Thus, FA shows potential for application as an antimicrobial compound against most important pathogenic microorganisms.

**Analytical methods**

The development of analytical methods for analyses is very relevant, mainly for monitoring the quality of marketed products (Marco *et al.* 2017). For this purpose, a review was conducted. Table II shows the different conditions and methods adopted for the analysis of FA.

HPLC is commonly used to analyze FA; however, the technique has some disadvantages. It involves the use of analytical columns and expensive equipment, needs a large volume of solvents in the mobile phase, and is associated with a high maintenance cost (Marco *et al.* 2017); nevertheless, it is one of the most commonly used separation techniques (Tótoli and Salgado, 2018).

As evident in Table II, the different methods of analysis are associated with toxic solvents such as methanol, trifluoroacetic acid and acetonitrile, which can be harmful to the environment. Thus, green solvents such as ethanol and water, are better options, because they are environmentally friendly, pose a lower risk to the operators' health, and are often associated with low costs (Gałuszka *et al.*, 2012; Zimmermann *et al.*, 2017).

Different flow rates, wavelengths, and columns have been used for FA analysis. pH adjustments for the mobile phase were performed using different solvents such as sodium hydroxide and glacial acetic acid.

| Method | Conditions | Detection system | Reference |
|--------|------------|------------------|-----------|
| HPLC   | C18 column (10 mm × 240 mm × 5 µm). Mobile phase: 0.01% acetic acid containing 28% methanol. pH 3.0. Flow rate: 1.0 mL.min⁻¹. | UV (310 nm) | Kroon *et al.* (1997) |
|        | C18 column (15 cm × 4.6 mm × 5 µm). Mobile phase: 0.01 M sodium citrate containing 13% methanol. pH 5.4. Flow rate: 1.0 mL.min⁻¹. Rt: 10.0 min. | UV (280 nm) | Zupfer *et al.* (1998). |
|        | ODS- Hypersil Column (10 mm × 4.6 mm × 5 pm). Mobile phase: a mixture of 12% methanol and a sodium citrate buffer. pH adjusted to 2.6 with sodium hydroxide solution. Flow rate: 1.0 mL.min⁻¹. | UV/Vis (320 and 418 nm) | Pussayanawin *et al.* (1988). |
|        | C8 column (4,6 × 250 mm). Mobile phase: 0.1% trifluoroacetic acid and 80% aqueous acetonitrile supplemented with trifluoroacetic acid. Flow rate: 1.0 mL.min⁻¹. | UV (254 nm) | Muheim; Lerch (1999) |
|        | Column Develosil ODS-HG-5 (RP-18, 250 × 20 mm). Mobile phase: methanol containing 1 mM trifluoroacetic acid (TFA). Flow rate: 7.0 mL.min⁻¹. Rt: 64 min. | UV (320 nm) | Andreasen *et al.* (2000) |

(continuing)

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2 Table II: Methods using FA. Abbreviations: FA, ferulic acid; HPLC, high-performance liquid chromatography; UV, ultraviolet; Vis, visible; Rt, retention time; LC, liquid chromatography; MS, mass spectrometry.
TABLE II - Analytical methods described in literature for the determination of ferulic acid.²

| Method     | Conditions                                                                 | Detection system     | Reference          |
|------------|-----------------------------------------------------------------------------|----------------------|--------------------|
| HPLC       | C18 Column Hypersil (200 × 4.6 mm × 5 µm). Mobile phase: a mixture of acetonitrile-water (16:84 v/v) containing 1% glacial acetic acid. Flow rate: 0.8 mL.min⁻¹. | UV (320 nm)          | Li; Bi (2003).     |
|            | Column HC-C18 (150 mm × 4.6 mm × 5 µm) with a guard C18 column. Mobile phase: a mixture of methanol-water containing 0.5% (v/v) of glacial acetic acid. Flow rate: 0.8 mL.min⁻¹. Rt: 8 min. | UV (281 nm)          | Li et al. (2007).  |
|            | BDS-Hypersil C18 column (250 mm × 4.6 mm × 6 µm). Mobile phase: 80% (v/v) acetonitrile/water. Flow rate: 1.0 mL.min⁻¹. Rt: 3.37 min. | UV (323 nm)          | Flores et al. (2016) |
| HPLC-CV    | C18 KinetexTM column (100 mm × 4.6 mm × 2.6 µm) with a guard C18 column (4.0 mm × 3.0 mm). Mobile phase: 0.06% of acetic acid and acetonitrile. pH adjusted to 6.0 with sodium hydroxide or acetic glacial acid. Flow rate: 1.0 mL.min⁻¹. | CV (−1.0 V to 1.2 V) | Natale et al. (2015) |
| LC-MS-MS   | C18 Column (250 mm × 4.6 mm × 5 µm) with a suitable guard C18 column (5 µm, 7.5 mm × 4.6 mm). Mobile phase: 1.0% acetic acid in water and acetonitrile. Flow rate: 1.0 mL.min⁻¹. | UV/DAD (320 nm)      | Lu et al. (2005).  |
|            | APCI-MS (positive and negative ion mode)                                     |                      |                    |
| UPLC       | C18 column (100 mm × 2.1 mm × 1.7 µm). Mobile phase: A mixture of 0.1% formic acid and acetonitrile. Flow rate: 0.4 mL.min⁻¹. | Triple quadrupole MS with ESI | Huang (2014)  |

Cytotoxicity

Besides the beneficial effects of FA, it is also important to know the cytotoxicity of the compound to ensure its safety on human skin. Many authors have investigated the effects of FA on human cells. Ogiwara and coworkers (2002) studied the cytotoxic activities of eugenol and FA on RAW 264.7 cells and found that they were similar to that in the human submandibular gland carcinoma cells, and that the cytotoxicity of FA was approximately 10-fold lower than that of eugenol. The number of moles of peroxy radical trapped by FA and eugenol was investigated, using the induction period methods of the methyl methacrylate polymerization system. FA had a higher number of moles of peroxy radical trapped than eugenol, similar to that of 2, 6-di-t-butyl-4-methylphenol. These results suggest that FA may be useful for preventing cell damage likely caused by $O_2^-$, and in particular by OH and NO, in living systems.

Peng and coworkers (2013) studied the cytotoxicity of FA (2 mM) against a bladder cancer cell line (T24 cells) cultured in 2D and 3D systems. Its toxicity in the 3D system was much higher than that in the 2D culture. The results encourage the use of FA in the treatment of this type of cancer.

Nanofibers containing FA showed cytotoxicity against hepatocellular carcinoma (HCC) cells (HepG2 cells) in the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. HCC is globally recognized as a major form of cancer. However, the inherent resistance of cancer cells to chemotherapy, and the augmented level of multi-drug resistance protein are some of the prime obstacles in HCC treatment. FA is a common phenolic phytochemical with anticancer activity against breast cancer, colon cancer,
skin cancer, and pulmonary cancer. A study reported that polymeric nanofibers without FA did not inhibit cell growth. On the other hand, cell growth inhibition rates of 51.9% and 71.3% (p < 0.01) were recorded for free FA and FA-encapsulated nanofibers, respectively (Vashisth et al., 2015).

A group of researchers investigated the protective effects of FA and related polyphenols against the cytotoxicity of glyoxal (GO) or methylglyoxal (MGO) in isolated rat hepatocytes. GO and MGO are substances that produce reactive oxygen and carbonyl species in the human body, leading to oxidative stress and damage to nucleic acids and proteins. Their effects can contribute to complications associated with diabetes mellitus, cardiovascular disease, and Alzheimer’s and Parkinson’s diseases. They found that the polyphenols significantly decreased reactive oxygen species formation and GO- or MGO-induced cytotoxicity in the test model (Al Maruf et al., 2015).

A cytotoxicity study showed that FA (~300 µg.mL⁻¹) did not cause any significant toxicity in platelets, leukocytes, and erythrocytes (Choi et al., 2018). The in vitro cytotoxicity of FA was also determined on CCK-8 cell line, and FA was nontoxic but was found to be toxic at a higher concentration (50 µM) (Nile et al., 2016). FA did not show cytotoxicity against human hepatoma (HepG2) and keratinocyte (HaCaT) cells (Nazaré, 2013).

CONCLUSIONS

This review provides information about the potential of FA in cosmetic and pharmaceutical preparations. FA has antioxidant potential, which is attributable to its structural characteristics, and antimicrobial activity, at variable intensity, against several fungal and bacterial strains. For the identification and quantification of FA, the most used analytical method is HPLC; however, the method is not eco-friendly. Furthermore, FA can protect cell lines against oxidation. However, it has a cytotoxic effect against cancer cell cultures.

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