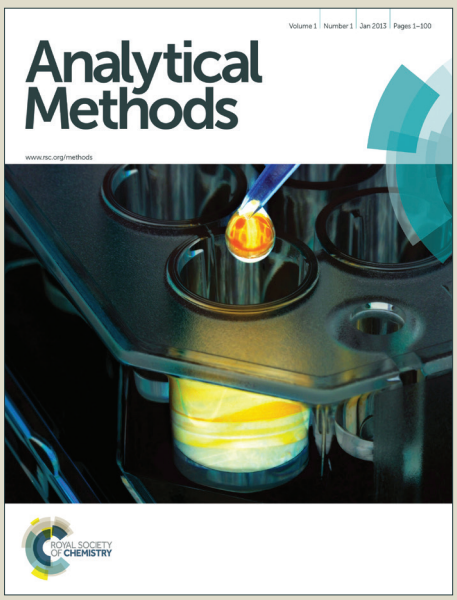
Analytical Methods

Accepted Manuscript



ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/methods

Analytical Methods

RSCPublishing

MINIREVIEW

Cite this: DOI:

Caffeic Acid: a review of its potential use for medications and cosmetics

Received
Accepted

DOI:

www.rsc.org/

C. Magnani,[a] V.L.B. Isaac,[a] M.A. Correa [a] and H.R.N. Salgado[a]

Besides powerful antioxidant activity, increasing collagen production and prevent premature aging, caffeic acid has demonstrated antimicrobial activity and may be promising in the treatment of dermal diseases. The relevance of this study is based on the use of caffeic acid increasingly common in humans. Thus, studies that demonstrate and clarify the functions of this substance are very important.

Introduction

Characteristics of caffeic acid

Phenolic compounds occur universally in the plant kingdom and are part of a large and complex group of organic substances. Higher plants synthesize and accumulate a wide variety of phenolic compounds, which confer protection against the attacks of free radicals, which are by products from the process of photosynthesis and against tissue injuries.[1] The phenolic compounds can be classified into two groups: group of simple phenolic compounds and group of polyphenolic compounds which can be observed through the frame shown in Figure 1.[2,3]

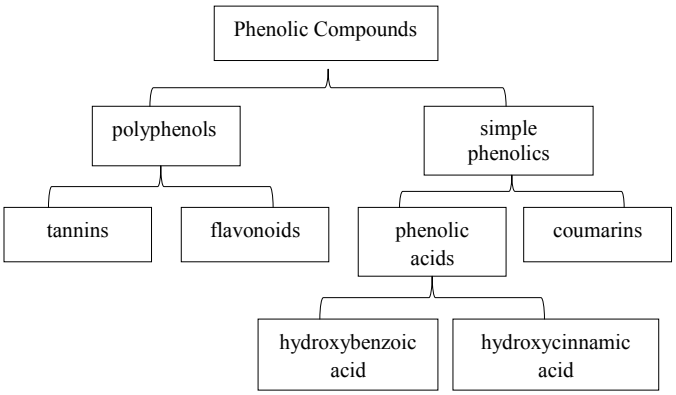


Fig. 1 Chemical classification of phenolic compounds.

Cinnamic acid derivatives, also called phenylpropanoids are nine carbon structures (Figure 2). Caffeic acid (3,4-dihydroxycinnamic) is representative of this group.

[a] UNESP - Universidade Estadual Paulista, Faculdade de Ciências Farmacêuticas, Departamento de Fármacos e Medicamentos– Araraquara, SP, Brasil.

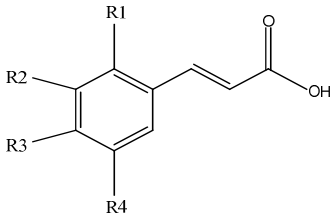


Fig. 2 Chemical structure of the main cinnamic acids. Cinnamic acid R1 = R2 = R3 = R4 = H, *o*-coumaric acid: R2 = OH, p-coumaric acid: R3 = OH; caffeic acid: R2 = R3 = OH; ferulic acid: R2 = OCH3 and R3 = OH.

Modern consumers increasingly demand products and foods with high quality. Cosmetics may not have as many health implications as pharmaceutical products or food, but they are chemical products that people use daily and apply on skin, hair, nails and even teeth or mouth.[4] Allied to this, legislation is increasingly strict about quality and safety, which has challenged the industries.[5] For this reason, natural antimicrobial compounds, such as polyphenols[6] are being widely used.

Caffeic acid (3,4-dihydroxycinnamic) is one of the hydroxy-cinnamate and phenylpropanoids metabolites more widely distributed in plant tissues. This polyphenol is present in many food sources, including coffee drinks, blueberries, apples and cider[7]. Besides food, caffeic acid is present in several medications of popular use, in the majority, based on propolis.[8] Besides acting as a carcinogenic inhibitor[9,10], is also known as an antioxidant and antibacterial in vitro, and can contribute to the prevention of atherosclerosis and other cardiovascular diseases[11-13].

Antioxidant Activity

Antioxidants are compounds that act by inhibiting or reducing the effects triggered by free radicals and oxidizing compounds.[14]

The phenolic antioxidants act as free radical scavengers and sometimes as metal chelators. They act both in the initiation step such as on the propagation of the oxidative process. The products are relatively stable due to the resonance of the aromatic ring shown by these substances. The phenolic acids are characterized by the presence of a benzene ring, a carboxylic acid grouping and one or more hydroxyl or methoxy groups of the molecule, which confers antioxidant properties.[15]

The phenylpropanoids act as antioxidants by eliminating oxygen free radicals[16,17] and chelating pro-oxidant metal ions, especially iron.[18,19] The hydroxyl groups of these molecules confer antioxidant activity, but they are not the only factors in determining the potency of their activities. There is a single hydroxyl group *para*-substituted in an aromatic ring that is linked to a side chain conjugate in the case of ferulic acid. This substitution allows the phenoxy radical free electrons to become delocalized over the entire molecule, and therefore stable.[20] The *ortho* substitution to the methoxy group, an electron donor, is also a contributing factor to the stability of the phenoxy radical and thus increases the efficiency antioxidant.[21,22] The presence of a second hydroxyl group in the *ortho* position, besides the *para* position is known to increase the antioxidant activity due to a additional resonance stabilization and formation of *o*-quinone (Figure 3).[20,21,23] This characteristic can be used to explain the fact that the efficiency of antioxidants such as caffeic acid is greater than that of ferulic acid.

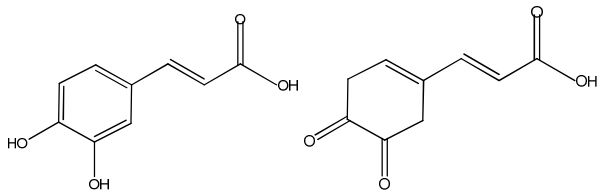


Fig. 3 Chemical structure of caffeic acid and formation of *ortho*-quinone.

Caffeic acid has been shown to be a α-tocopherol protective in low-density lipoprotein (LDL).[24] Furthermore, their combination with other products, such as with chlorogenic and caftaric acids showed more potent antioxidant activity in a variety of different systems.[17,25]

Natural phenolic antioxidants including caffeic and ferulic acids, gained remarkable attention as promising photoprotective agents[26,27] and also have been present in skin care products for its antioxidant activity. However the literature shows little evidence about the usefulness of hydroxycinnamic acids to protect the skin from photo-oxidative damage.

The normal cellular metabolism produces free radicals, including reactive species of oxygen and nitrogen, which are derived from both normal metabolic processes and essential metabolic processes to the body (endogenous), and they can be derived from exposure to environmental factors (exogenous) such as pollution, radiation, pesticides tobacco, among others. Free radicals can cause beneficial or deleterious effects to health.[28-30]

The importance of reactive oxygen species (ROS) and free radicals has attracted increasing attention over the last decade. ROS, which include free radicals such as superoxide anion radicals ($O_2^{\bullet-}$), hydroxyl radicals ($OH^{\bullet}$) and reactive oxygen species such as H_2O_2 and singlet oxygen ($1O_2$). These molecules exacerbate factors of cell damage and aging.[31,32]

ROS are continuously produced during normal physiologic events and they can easily initiate the peroxidation of membrane lipids, leading to the accumulation of lipid peroxides.[33,34] However antioxidants cells have developed mechanisms to protect themselves from free radical toxicity. The agents considered antioxidants include: catalytic enzymes that remove radicals such as superoxide dismutase (SOD); proteins which minimizes the availability of pro-oxidants, such as iron ions and copper ions, for example, transferrins, ferritins, metallothionein and haptoglobines; low molecular weight molecules that have the ability to capture reactive oxygen species through autoxidation such as, for example, those with glutathione and thiol group (SH) or vitamins such as α-tocopherol, ascorbic acid and β-carotene.[35,36]

When there is an imbalance between pro and antioxidant system, with a predominance of oxidants, the oxidative stress occurs [37,38]. This stress can be associated with damage to lipids, proteins and gens and it imply a great variety of human diseases as well as in the aging process.[39]

The free radicals also act on the skin tissue, which originate mainly by exogenous actions, such as ultraviolet radiation. Depending on the dose, exposure time, wavelength and area exposed, the ultraviolet radiation can cause skin burns, premature skin aging and even skin cell DNA damage and skin cancer.[40,41]

Thus, the natural aging of the skin associated with the action of free radicals causes a reduction in skin hydration, pigmentation, fine wrinkles, signals from sagging and increased possibility of the occurrence of neoplasm diseases. Another change is the reduction of dermal collagen that makes the skin thinner. These changes are even more evident after menopause, in which there is a rapid decrease in the levels of collagen in skin and bone, suggesting that estrogen influences the collagen synthesis in the skin as much bone mass.[42]

In order to maintain the skin healthier and younger-looking, thereby mitigating the effects of aging, more strategies have been suggested.[30] The use of natural or synthetic antioxidants in foods, cosmetics, beverages and also medicine is one of the defence mechanisms against free radicals.[43-45]

Photoaging of the skin is one of the most common dermatological concerns and can become a major health concern because it is correlated with an increased risk of skin cancer.[42] UVA radiation can cause damage clinical, biochemical and histological changes in the skin through

changes in cells and in extracellular matrix proteins, including collagen, responsible for the structural integrity of the skin.[46]

UVA radiation (315-400 nm) has been shown to elevate matrix metalloproteinase-1 extracellular (collagenase MMP-1), most proteolytic enzyme produced by the skin cells, including keratinocytes and fibroblasts.[47,48] This activation is related to oxidative stress, which occurs when there is excessive production of reactive oxygen species and / or depletion of antioxidant defences in cells.[49,50]

Natural and synthetic antioxidants act as scavenger of free radicals and have been shown to promote skin photo protection by repealing the induction of MMP-1 activity mediated by UVA radiation and expression of keratinocytes and skin fibroblasts.[48,51,52]

Ultraviolet A (UVA) plays a fundamental topic in the pathogenesis of premature skin aging through cytotoxicity to keratinocytes and degradation of collagen, a major component of the extracellular matrix that provides structural support. Protection against UVA damage mediated through the antioxidant defence systems has been proposed as a possible mechanism by which plant compounds decrease the process of premature aging.[53]

Furthermore, under stimulation by UVA and UVB sunlight, keratinocytes from human skin can secrete nitric oxide (a radical involved in oxidation reactions). Nitric oxide (NO) appears to have great interest in the formation of erythema and skin inflammation.[54,55] Both caffeic and ferulic acid act as sequestering radical NO.[56] In light of these observations, the topical administration of antioxidants found considerable interest, since it represents an effective strategy to protect the skin against oxidative damage mediated by UV.[55,57]

The extension of erythema caused by UVB radiation may be monitored through reflectance spectrophotometry, which is considered one of the most suitable models for the study *in vivo* of skin damage after acute exposure to UV.[58,59] Caffeic and ferulic acids, dissolved in saturated aqueous solutions at pH 7.2, proved to provide significant photo-protection to the skin against UV-induced erythema.[56]

Anti-aging products are being increasingly appreciated in recent years and those with active antioxidants in its composition are highlighted.[60]

Caffeic acid was an effective antioxidant in different methods for the determination of antioxidant activity *in vitro*, including total antioxidant activity of the ferric thiocyanate method, method of inhibition of ABTS•+ and DPPH•, the superoxide anion radical and metal chelating activity when compared with conventional antioxidants such as BHA, BHT, α-tocopherol, a natural antioxidant and Trolox, which is a water soluble analogue of tocopherol.[61]

Through the permeation study of caffeic acid, chlorogenic acid and oraposide, it was found that the caffeic and chlorogenic acids are able to permeate all the skin layers of pig ear, which have a systemic activity, whereas oraposide remained in the upper layer of the skin surface.[62] Permeation studies with human skin also confirm the ability of caffeic acid and ferulic acid permeate into the skin, characteristic independent of the pH of the receptor solution being 3.0 or 7.2.[63]

As the solar radiation penetrates deeply into the skin, it is necessary to ensure that topically applied substances are able to penetrate through the corneum stratum, the main barrier against permeation of foreign substances through the skin and reach the deepest layers to promote satisfactory photo-protection.[64]

The absorption of a compound in the skin is determined by its physicochemical characteristics, and on the permeation process, the lipophilicity is one of the most important features.[65] Thus, the high lipophilicity of caffeic and ferulic acid may explain the fact to permeate through the stratum corneum.[66]

The ferulic and caffeic acid also demonstrate efficiency to protect phosphatidylcholine peroxidation induced by UV radiation, which is important since the phosphatidylcholine is a major constituent of the lipid bilayers of cell membranes.[56]

Antimicrobial Activity

Every day more consumers seek more effective cosmetics, which approximate the definition of cosmeceuticals, or actually having some biological effect, particularly in the prevention of premature aging and diseases such as cancers caused by the action of free radicals in the genes of cells. Therefore, as well as drugs, these products should also provide security to the consumer. Allied to this, legislation is becoming increasingly stringent, which has challenged the industries.[4,5] Besides, cosmetic companies are required to control the optimal preservation of their commercial products, since microbial contamination in cosmetics represents an important risk for consumer health.[67] For this reason, natural antimicrobial compounds are widely used.[6]

Besides its remarkable antioxidant activity, *in vitro* studies have demonstrated antimicrobial properties of propolis against various oral pathogens. Several components of propolis have been analysed in different countries, and caffeic acid, phenethyl ester of caffeic acid and flavonoids are the main ingredient responsible for the antibiotic power of this resin.[68-70] Thus, a thorough search of the antimicrobial activity of caffeic acid is promising targeting the treatment of dermal diseases, such as acne.

Some phenylpropanoids, including caffeic acid, *p*-coumaric acid and ferulic acid are able to inhibit the growth of bacteria, including *E. coli*, *Staphylococcus aureus*, *Bacillus cereus*, *Listeria monocytogenes* and some yeasts.[71]

The antimicrobial activity of these substances depends not only on its structure but also on the environmental conditions. Caffeic acid has a reduced ability to inhibit *Listeria* compared to other hydroxycinnamic acid derivatives due to the presence of a high degree of hydroxylation.[6] Moreover, environmental factors, including pH and concentration of sodium chloride are important because they produce certain physiological changes in the micro-organisms that make them more sensitive to phenylpropanoids.[72]

Antioxidant and antimicrobial activities of caffeic acid is pronounced in cosmetic emulsions with acidic pH (3 e 5). Caffeic Acid showed in this pH, antimicrobial effects against various micro-organisms studied (*E. coli*, *Pseudomonas aeruginosa*, *Bacillus cereus*, *Kokuria rhizophila*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Candida albicans*).[73]

The mechanism of antimicrobial activity of substances is established considering one of the following three: (1) reaction with the cell membrane causing increased permeability and loss of cellular constituents, (2) inactivation enzymatic systems or essential enzymes, including those involved in the production process energy and synthesis of structural components, or (3) destruction or inactivation functional genetic material.[74-75]

The cytoplasm membrane of bacteria and mitochondrial membrane of yeasts are a permeable barrier to passage of small ions such as H^+, K^+, Na^+ and Ca^{++}, in addition to being responsible for input and output of different compounds. This cell permeability is important for various cell functions such as maintenance of energy in the transduction process, solute transport, metabolism regulation and pressure control.[76-77] There is a consensus that aromatic and phenolic compounds, such as caffeic acid, affect the cytoplasm membrane, alter their structure and function, change the active transport and coagulate cell content. [78-79]

Cytotoxicity

Caffeic acid and its derivatives, such as caffeic acid phenyl ester, has action against colon and oral cancer, and they are inhibitors of cyclooxygenase II (COX-2).[80-82] These substances are cytotoxic for tumours but not for normal cells.[83-84]

An analysis by flow cytometry showed that the caffeic acid and its derivatives caused cellular entrapment of oral squamous cell carcinoma cells, Meng-1 (OEC-M1) in G2/M phase of cell division. These differential effects on cancer show that these compounds may be useful in cancer chemotherapy oral.[85]

Various natural antioxidants were tested, among them caffeic acid, catechin, epicatechin and quercetin to evaluate the reduction of the cytotoxicity induced by peroxynitrite in chondrosarcoma cells. This observation suggests that diets rich in antioxidant compounds are able to limit cell cytotoxicity.[86]

A derivative of caffeic acid, the caffeic acid phenyl ester was used and it was observed that it inhibited growth and secretion of interleucin-6, and it induced apoptosis in a dose and time dependent on multiple myeloma cells ARH-77. Therefore, both the caffeic acid as its derivative deserves further study as an effective agent against multiple myeloma.[87]

Treatment of mice with propolis, caffeic acid phenyl ester and caffeic acid itself, significantly reduced the number of tumour nodules in the lungs of animals whose lung nodules were generated by injection of viable tumour cells intravenously. In in vitro studies, the propolis did not affect tumour cell growth, while the phenyl ester of caffeic acid and caffeic acid expressed a strong cytotoxicity to cells.[88]

The p38, a mitogen-activated protein kinase (MAPK) responds to stimuli such as heat, shock, cytokines, UV and is directly involved in cell proliferation and production of NO.[89] Caffeic acid significantly reduces mRNA expression of Interleucin-10 UVB-induced in murine and also inhibits the activation of p38-MAPK.[90] The caffeic acid also reduces the migratory capacity of malignant keratinocytes.[91]

Although many studies demonstrate the antitumoral activity of caffeic acid, others show opposite effects. The carcinogenicity of low dietary levels of the antioxidants caffeic acid, butylated hydroxyanisole (BHA), sesamol, 4-methoxyphenol (4-MP) and catechol, were examined alone or in combination in a 2-year long-term experiment. The results indicate that even at low dose levels phenolic compounds can exert additive/synergistic effect on carcinogenesis. [92] Furthermore, caffeic acid is also listed under some Hazard Data and in the International Agency for Research on Cancer in the Group 2B, as a substance "possibly carcinogenic to humans".

As there are many controversial results about the protective or carcinogenic potential of caffeic acid, more studies should be conducted to elucidate its therapeutic use.

Analytical Methods

There is no method for determination and quantification of caffeic acid in official compendia, such as pharmacopoeias, however most current articles recommends quantification of the substance by High Performance Liquid Chromatography (HPLC).

For determination of rosmarinic and caffeic acids in various herbs such as rosemary, sage, thyme, mint, lemon balm and lavender, a HPLC method was developed and validated. The separation system consisted of a reversed phase C18 column, a gradient elution system of methanol/water containing phosphoric acid and a photodiode array detector. The method proved to be simple, sensitive, reproducible and fast ideal for routine analysis.[93]

For quantitation of receptor solution in a permeation study, the chromatography was performed on a Hypersil ODS column (particle size: 5 µm x 25 cm x 4.0 mm). The mobile phase was acetonitrile-water (18:82) containing 2% acetic acid. The flow rate was set at 1.0 mL/min. Each sample was filtered before injection using a Millex filter and an aliquot (20 mL) was injected into HPLC. Detection was performed at 302 nm.[63]

Marti-Mestres and coworkers (2007) conducted a permeation study by applying 50 µL of test formulation 2% caffeic acid in propyleneglycol-transcutol (1:1) on the skin of the pig's ear and used isotonic saline (NaCl 0.9%) with 1% gentamycin pH 7.0 as the receiver solution. This solution was maintained at 37ºC and under these conditions the skin temperature is 32ºC, which corresponds to the temperature of the body surface *in vivo*. The quantification of the receptor solution was also carried out by HPLC with UV detection, column C-8 reverse phase (5 µm, 250 mm x 3 mm) at 40ºC. The mobile phase was acetonitrile-water (18:80) with 2% acetic

acid and flow rate of 0.5 mL/min. Detection was at 330 nm and the retention time was approximately 6.0 min.[62]

Chromatographic techniques are widely used in the separation, purification, identification and quantification of substances. Although HPLC is a robust method, it is more suitable for mixtures with known compositions. For identification of unknown substances in complex samples the most suitable method is MS or LC-MS/MS. Many papers have been published in recent years on the examination of caffeic acid by LC-MS/MS approaches.

An example of identification of caffeic acid in a complex matrix was done for simultaneous analysis of alkamides and caffeic acid derivatives from *Echinacea purpurea* extracts. The analysis was carried out with reversed phase HPLC coupled to electrospray ionization mass spectrometry (ESI-MS). The flow rate was set to 0.2 mL/min and the eluents were A = water + 1% acetic acid and B = acetonitrile). The gradient condition was: 90% of A in B for 4 min; 90–60% of A in B from 4 to 15 min; 60–40% of A in B from 15 to 30 min; 100% B from 30 to 35 min; 90% of A in B from 35 to 43 min. The outlet of the HPLC column was directly connected to the electrospray ionization source of an ion trap mass spectrometer. The mass spectrometer was operated in the negative ion mode the first 15 min of the analysis, then switched to the positive ion mode for the remainder. The total analysis time was 43 min. This new method was considered effective for the quality control of these extracts that requires rapid methods to determine their chemical composition. [94]

Another method was developed and validated to assess the qualitative and quantitative profiles of *Myrcia bella* hydroalcoholic extract. In total, 24 constituents were characterized, including phenolic acids such as caffeic acid, by means of extensive preparative chromatographic analyses, along with mass spectroscopy and Nuclear Magnetic Resonance (NMR) techniques. And it shows that the mass spectroscopy technique is a powerful tool for direct and rapid identification of the constituents after isolation and NMR characterization. [95]

Plasma is another type of complex matrix in which caffeic acid may be found. A validated method was developed for the simultaneous determination of the hydroxycinnamates caffeic acid, dihydrocaffeic acid, ferulic acid, dihydroferulic acid, and isoferulic acid in human plasma as metabolites derived from coffee consumption. It was possible using high-performance liquid chromatography coupled to negative electrospray ionization tandem mass spectrometry. [96] These same techniques were used in conjunction to develop a simple, rapid and sensitive method for the simultaneous quantification of chlorogenic acid and caffeic acid in rat plasma. [97]

Conclusions

This review was carefully prepared to prove the potential of caffeic acid use in cosmetics and pharmaceutical preparations and to encourage more studies to elucidate the activity of this substance on the human body.

Acknowledgements
FAPESP, CNPq and PADC-FCF-UNESP.

References

1 S.E. Soares, Ácidos fenólicos como antioxidantes, *Rev. Nutr.*, 2002, 15, pp.71-81.
2 E.A. Melo and N.B. Guerra, Ação antioxidante de compostos fenólicos naturalmente presentes em alimentos, *Bol. SBCTA*, 2002, 36, pp.1-11.
3 J. Burns, P.T. Gardner, D. Matthews, G.G. Duthie, M.E. Lean and A. Crozier, Extraction of phenolics and changes in antioxidant activity of red wines during vinification, *J. Agric. Food Chem*., 2001, 49, pp. 5797-5808.
4 A. Chisvert and A. Salvador, Cosmetic ingredients: from the cosmetic to the human body and the environment, *Anal. Methods,* 2013, 5, pp. 309.
5 A.I. Nazer, A. Kobilinsky, J.L. Tholozan and F. Dubois-Brissonnet, Combinations of food antimicrobials at low levels to inhibit the growth of *Salmonella sv. typhimurium*: a synergistic effect?, *Food Microbiol*., 2005, 22, pp. 391–8.
6 A.M. Wen, P. Delaquis, K. Stanich and P. Toivonen, Antilisterial activity of selected phenolic acids, *Food Microbiol*., 2003, 20, pp. 305–11.
7 M.N. Clifford, Chlorogenic acids and other cinnamates- nature, occurrence, dietary burden, absorption and metabolism. *J. Sci. Food Agric.,* 2000, 80, pp. 1033–43.
8 S.R. Lustosa, A.B. Galindo, L.C.C. Nunes, K.P. Randau and P.J. Rolim Neto, Propolis: atualizações sobre a química e a farmacologia. *Rev. Bras. Farmacogn*., 2008, 18, pp.447-454.
9 M.T. Huang and T. Ferraro, Phenolic-compounds in food and cancer prevention, *Acs Symposium Series*, 1992, 507, pp.8–34.
10 P. Greenwald. Clinical trials in cancer prevention: current results and perspectives for the future, *J. Nutr*., 2004, 134, pp.3507S-3512S.
11 C. Sanchez-Moreno, A. Jimenez-Escrig and F. Saura-Calixto, Study of low-density lipoprotein oxidizability indexes to measure the antioxidant activity of dietary polyphenol, *Nutr. Res*., 2000, 20, pp.941–53.
12 J.A. Vinson, K. Teufel and N. Wu, Red wine, dealcoholized red wine, and especially grape juice, inhibit atherosclerosis in a hamster model, *Atherosclerosis*, 2001, 156, pp. 67–72.
13 Worldwide Chemical Information, Trading & Advertising. Available in: http://www.chemicalland21.com/lifescience/phar/caffeic%20acid.htm> accessed in: Feb. 1st 2014.
14 D.G. Soares, A.C. Andreazza and M. Salvador, Avaliação de compostos com atividade antioxidante em células da levedura *Sacharomyces cerevisae*, *Rev. Bras. Ciênc. Farm*., 2005, 1, pp.95-100.
15 Dossiê de Antioxidantes. Os Antioxidantes. Available in: <http://www.revista-fi.com/materias/83.pdf.> Accessed in Feb. 1st 2014.
16 J.H. Chen, C.T. Ho, Antioxidant activities of caffeic acid and its related hydroxycinnamic acid compound, *J. Agric. Food Chem.*, 1997, 45, pp. 2374–8.

17 A.S. Meyer, J.L. Donovan, D.A. Pearson, A.L. Waterhouse and E.N. Frankel, Fruit hydroxycinnamic acids inhibit low density lipoprotein oxidation in vitro, *J. Agric. Food Chem*., 1998, 46, pp.1783–1787.

18 F. Paiva-Martins and M.H. Gordon, Effects of pH and ferric ions on the antioxidant activity of olive polyphenols in oil-in-water emulsions, *J. Amer. Oil Chem. Soc.*, 2002, 79, pp.571–6.

19 M.J. Hynes and W.O. Coinceanainn, The kinetics and mechanisms of reactions of iron(III) with caffeic acid, chlorogenic acid, sinapic acid, ferulic acid and naringin, *J. Inorg. Biochem.*, 2004, 98, pp.1457–64.

20 E. Graf, Antioxidant potential of ferulic acid. *Free Radical Biol. Med*., 1992, 13, pp.435-448.

21 M.E. Cuvelier, H. Richard and C. Berset, Antioxidative activity and phenolic composition of pilot-plant and commercial extracts of sage and rosemary, *J. Am. Oil Chem. Soc*., 1996, 73, pp. 645-652.

22 J. Terao, H. Karasawa, H. Arai, A. Nagao, T. Suzuki and K. Takawa, Peroxyl radical scavenging activity of caffeic acid and its related phenolic compounds in solution, *Biosci. Biotechnol. Biochem.*, 1973, 57, pp.1204-1205.

23 W. Brand-Willams, M.E. Cuvelier and C. Berset, Use of free radical method to evaluate antioxidant activity. Food Sci.Technol., 1995, 28, pp.25-30.

24 J. Laranjinha, O. Vierira, L. Almeida and V. Madeira, Inhibition of metmyoglobin/H2O2-dependent low density lipoprotein lipid peroxidation by naturally occurring phenolic acids, *Biochem. Pharmacol*., 1996, 51, pp.395-402.

25 L.R. Fukumoto and G. Mazza, Assessing antioxidant and prooxidant activities of phenolic compounds, *J. Agric. Food Chem*., 2000, 48, pp. 3597–3604.

26 Y.Yamada, H. Yasui and H. Sakurai, Suppressive effect of caffeic acid and its derivatives on the generation of UVA-induced reactive oxygen species in the skin of hairless mice and pharmacokinetic analysis on organ distribution of caffeic acid in ddY mice, *Photochem. Photobiol*., 2006, 82, pp.1668–1676.

27 J.C. Murray, J.A. Burch, R.D. Streilein, M.A. Iannacchione, R.P. Hall and S.R. Pinnell, A topical antioxidant solution containing vitamins C and E stabilized by ferulic acid provides protection for human skin against damage caused by ultraviolet irradiation, *J. Am. Acad. Dermatol*., 2008, 59, pp. 418–425.

28 M.I. Hadshiew, M.S. Eller and B.A. Gilchrest, Skin aging and photoaging: the role of DNA damage and repair. *Am. J. Contact Dermat.*, 2000, 11, pp.19-25.

29 M. Yaar and B.A. Gilchrest, Skin aging: Postulated mechanisms and consequent changes in structure and function, *Clinics in Geriatric Medicine*, 2001, 17, pp. 617-630.

30 K. G. Menon, L. Dryer and R. Kalafsky, Aproaches to the development of cosmetic products to counter the effects of skin aging, *Skin Aging Handbook*, 2009, pp.265-290.

31 B. Halliwell and J.M. Gutteridge, *Free Radicals in Biology and Medicine,* Clarendon Press, Oxford, New York, 2007.

32 I. Gülçin, M.E. Büyükokuroglu, M. Oktay and Ö.I. Küfrevioglu, On the in vitro antioxidant properties of melatonin, *J. Pineal Res*., 2002, 33, pp.167–171.

33 P.D. Duh, Y.Y. Tu and G.C. Yen, Antioxidant activity of water extract of harng jyur (*Chrysanthemum morifolium* Ramat), *Lebens. Wiss. Technol*., 1999, 32, pp.269–277.

34 M.E. Büyükokuroglu, I. Gülçin, M. Oktay and Ö. I. Küfrevioglu, In vitro antioxidant properties of dantrolene sodium, *Pharmacol. Res.*, 2001, 44, pp.491–495.

35 E. Cadenas, Biochemistry of oxygen toxicity, *Annu. Rev. Biochem*., 1989, 58, pp.79-110.

36 C.M. Berra, C.F.M. Menck and P. Di Mascio, Estresse oxidativo, lesões no genoma e processos de sinalização no controle do ciclo celular, *Quim. Nova*, 2006, 29, pp.1340-1344.

37 F.Q. Schafer and G.R. Buettner, Redox environment of the cell as viewed through the redox state of the glutathione disulfide/glutathione couple, *Free Radic. Biol. Med*., 2001, 30, pp.1191-212.

38 T. Finkel and N.J. Holbrook, Oxidative Stress and Aging: Catalase Is a Longevity Determinant Enzyme, *Nature*, 2000, 408, pp.239.

39 M. Valko, D. Leibfritz, J. Moncol, M.T. Cronin, M. Mazur, J. Telser, Free radicals and antioxidants in normal physiological functions and human disease, *Int. J. Biochem. Cell Biol*., 2007, 39, pp.44-84.

40 K. Scharffetter-Kochanek, M. Wlaschek, P. Brenneisen, M. Schauen, R. Blaudschun and J. Wenk, UV-induced reactive oxygen species in photocarcinogenesis and photoaging, *Biol. Chemistry*, 1997, 378, pp.1247-1257.

41 Y. Matsumura and H.N. Ananthaswamy, Short-term and long-term cellular and molecular events following UV irradiation of skin: implications for molecular medicine, *Expert Rev. Mol. Med*., 2002, 2, pp.1-22.

42 M. Yaar and B.A. Gilchrest, Photo ageing: Mechanism, prevention and therapy, *Br. J. Dermatol*., 2007, 157, pp.874–877.

43 J.H. Doroshow, Effect of anthracycline antibiotics on oxygen radical formation in rat heart, *Cancer Research*, 1983, 43, pp.460-472.

44 B. Halliwell, R. Aeschbach, J. Lölinger and O.I. Aruoma, The characterization on antioxidants, *Food Chem. Toxicol*., 1995, 33, pp.601-617.

45 N.I. Weijl, F.J. Cleton and S. Osanto, Free radicals and antioxidants in chemotherapy-induced toxicity, *Cancer Treatment Reviews*, 1997, 23, pp.209-240.

46 G.J. Fisher, Z.Q. Wang, S.C. Datta, J. Varani, S. Kang and J.J. Voorhees, Pathophysiology of premature skin aging induced by ultraviolet light, *N. Engl. J. Med*., 1997, 337, pp. 1419–1428.

47 M. Brennan, H. Bhatti, K.C. Nerusu, N. Bhagavathula, S. Kang, G.J. Fisher, J. Varani and J.J. Voorhees, Matrix metalloproteinase-1 is the major collagenolytic enzyme responsible for collagen damage in UV-irradiated human skin, *Photochem. Photobiol*., 2003, 78, pp.43–48.

48 N. Philips, J. Smith, T. Keller and S. Gonzalez, Predominant effects of *Polypodium leucotomos* on membrane integrity, lipid peroxidation, and expression of elastin and matrixmetalloproteinase-1 in ultraviolet radiation exposed fibroblasts, and keratinocytes, *J. Dermatol. Sci*., 2003, 32, pp.1–9.

49 T. Polte and R.M. Tyrrell, Involvement of lipid peroxidation and organic peroxides in UVA-induced matrix metalloproteinase-1 expression, *Free Rad. Biol. Med.*, 2004, 36, pp. 1566–1574.

50 M.J. Pygmalion, L. Ruiz, E. Popovic, J. Gizard, P. Portes, X. Marat, K. Lucet-Levannier, B. Muller and J.B. Galey, Skin cell protection against UVA by Sideroxyl, a new antioxidant complementary to sunscreens, *Free Rad. Biol. Med.*, 2010, 49, pp.1629–1637.

51 E. Venditti, F. Bruge, P. Astolfi, I. Kochevar and E. Damiani, Nitroxides and a nitroxide-based UV filter have the potential to photo protect UVA-irradiated human skin fibroblasts against oxidative damage*, J. Dermatol. Sci*., 2011, 63, pp.55–61.

52 K. Wertz, N. Seifert, P.B. Hunziker, G. Riss, A. Wyss, C. Lankin and R. Goralczyk, Beta-carotene inhibits UVA-induced matrix metalloprotease 1 and 10 expression in keratinocytes by a singlet oxygen-dependent mechanism*, Free Rad. Biol. Med.,* 2004, 37, pp.654–670.

53 T. Pluemsamran, T. Onkoksoong and U. Panich, Caffeic acid and ferulic acid inhibit UVA-induced matrix metalloproteinase-1 through regulation of antioxidant defense system in keratinocyte HaCaT cells, *Photochem. Photobiol*., 2012, 88, pp.961–968.

54 G. Deliconstantinos, V. Villiotou and J.C. Stavrides, Nitric oxide and peroxynitrite skin erythema and inflammation, *Exp. Physiol.*, 1996, 81, pp.1021-1033.

55 C. Romero-Graillet, E. Aberdam, M. Clement, J.P. Ortonne and R. Ballotti, Nitric oxide produced by ultraviolet-irradiated keratinocytes stimulates melanogenesis, *J. Clin. Invest.*, 1997, 99, pp.635-642.

56 A. Saija, A. Tomaino, R. Lo Cascio, D. Trombetta, A. Proteggente, A. De Pasquale, N. Uccela and F. Bonina, Ferulic and caffeic acids as potential protective agents against photooxidative skin damage, *J. Sci. Food Agric*., 1999, 79, pp.476.480.

57 A. Saija, A. Tomaino, D. Trombetta, M. Giacchi, A. De Pasquale and F. Bonina, Influence of different penetration enhancers on 'in vitro' skin permeation and 'in vivo' photo protective effect of flavonoids, *Int. J. Pharm*., 1998, 175, pp.85-94.

58 J.B. Dawson, D.J. Barker, D.J. Ellis, E. Grassam, J.A. Catterill, G.W. Fischer and J.W. Feather, A theoretical and experimental study of light absorption and scattering by in vivo skin, *Phys. Med. Biol*., 1980, 25, pp.696–709.

59 P.H. Andersen, K. Abrams, P. Bjerring and H.A. Maibach, A time-correlation study of ultraviolet B-induced erythema measured by reflectance spectroscopy and laser Doppler flowmetry, *Photodermatol. Photoimmunol. Photomed*., 1991, 8, pp. 123–128.

60 II Caderno de Tendências, Higiene Pessoal, Perfumaria e Cosméticos. Available in: <http://www.abihpec.org.br/novo-wp/wp- content/uploads/2011/08/caderno_tendencias1.pdf.> Accessed in: Aug. 2013.

61 I. Gülçin Antioxidant activity of caffeic acid (3,4-dihydroxycinnamic acid), *Toxicology*, 2006, 217, pp.213–220.

62 G. Marti-Mestres, J.P. Mestres, J. Bres, S. Martin, J. Ramos and L. Viana, The "in vitro" percutaneous penetration of three antioxidant compounds, *Int. J. Pharm*., 2007, 331, pp.139–144.

63 A. Saija, A. Tomaino, D. Trombetta, A. De Pasquale, N. Uccella, T. Barbuzzi, D. Paolino and F. Bonina, In vitro and in vivo evaluation of caffeic and ferulic acids as topical photoprotective agents, *International Journal of Pharmaceutics*, 2000, 199, pp.39–47.

64 F. Bonina, M. Lanza, L. Montenegro, C. Puglisi, A. Tomaino, D. Trombetta, F. Castelli and A. Saija, Flavonoids as potential protective agents against photo-oxidative skin damage, *Int. J. Pharm.*, 1996, 94, pp.145-87.

65 J. Zatz, Percutaneous absorption, Wiley, New York, 1985.

66 S. Shahrzad and I. Bitsch, Determination of some pharmacology active phenolic acids in juices high-perfomance liquid chromatography, *J. Chromatogr. A*, 1996, 742, pp.223-231.

67 G. Alvarez-Rivera, T. Miguel, M. Llompart, C. Garcia-Jares, T. G. Villa and M. Lores, A novel outlook on detecting microbial contamination in cosmetic products: analysis of biomarker volatile compounds by solid-phase microextraction gas chromatography-mass spectrometry, *Anal. Methods*, 2013, 5, pp.384.

68 B. Havsteen, Flavonoids, a class of natural products of high pharmacological potency, *Biol Pharm.*, 1983, 32, pp.1141-1148.

69 M.C. Marcucci, Propriedades biológicas e terapêuticas dos constituintes químicos da própolis, *Quim. Nova*, 1996, 19, pp.529-535.

70 H. Menezes, Própolis: uma revisão dos recentes estudos de suas propriedades farmacológicas, *Arq. Inst. Biol.*, 2005, 72, pp.405-441.

71 F.M. Campos, J.A. Couto and T.A. Hogg, Influence of phenolic acids on growth and inactivation of *Oenococcus oeni* and *Lactobacillus hilgardii. J. Appl. Microbiol.*, 2003, 94, pp.167–74.

72 N. Canillac and A. Mourey, Effects of several environmental factors on the anti-*Listeria monocytogenes* activity of an essential oil of *Picea excels*, *Int. J. Food Microbiol.*, 2004, 92, pp.95–103.

73 M.P. Almajano, R. Carb'o, M.E. Delgado and M.H. Gordon, Effect of pH on the Antimicrobial activity and oxidative stability of oil-in-water emulsions containing caffeic acid, *J. Food Sci.*, 2007, 72, pp. C258-63.

74 A.L. Branen, Introduction to the use of antimicrobials. In: P.P. Davidson and A.L. Branen, *Antimicrobials in Foods*, Marcel Dekker, New York, 1993.

75 J.M. Kim, M.R. Marshall, J.A. Corneel, J.F. Preston and C.I. Wei, Antibacterial activity of carvacrol, citral and geraniol against *Salmonella typhimurium* in culture medium and fish cubes, *J. Food Sci.*, 1995, 60, pp. 1364- 1374.

76 I.R. Booth, Regulation of cytoplasmatic pH in bacteria, *Microbiol. Rev.*, 1985, 49, pp. 359-378.

77 S.D. Cox, C.M. Mann, J.L. Markham, H.C. Bell, J.E. Gustafson, J.R. Warmington and S.G. Wyllie, The mode of antimicrobial

action of essential oil of *Melaleuca alternifolia* (tea tree oil), *J. Appl. Microbiol.*, 2000, 88, pp.170-175.

78 J. Sikkema, J.A.M De Bont and B. Poolman, Mechanism of membrane toxicity of hydrocarbons, *Microbiol. Rev.*, 1995, 59, pp. 201-222.

79 S. Burt, Essential oils: theirs antibacterial properties and potential applications in foods- a review, *Int. J. Food Microbiol.*, 2004, 94, pp.223-253.

80 G.C. Ceschel, P. Maffei, A. Sforzini, B.S. Lombardi, A. Yasin and C. Ronchi, In vitro permeation through porcine buccal mucosa of caffeic acid phenethyl ester (CAPE) from a topical mucoadhesive gel containing propolis, *Fitoterapia*, 2002, 73, pp.44–52.

81 P. Michaluart, J.L. Masferrer, A.M. Carothers, K. Subbaramaiah, B.S. Zweifel and C. Kobolt, Inhibitory effects of caffeic acid phenethyl ester on the activity and expression of cyclooxygenase-2 in human oral epithelial cells and in a rat model of inflammation, *J. Cancer Res.*, 1999, 59, pp.2347–2352.

82 M.J Weyant, A.M. Carothers, M.E. Bertagnolli and M.M. Bertagnolli, Colon cancer chemo preventive drugs modulate integrin-mediated signaling pathways, *Clin. Cancer Res.*, 2000, 6, pp.949–956.

83 A. Russo, R. Longo and A. Vanella, Antioxidant activity of propolis: role of caffeic acid phenethyl ester and galangin, *Fitoterapia*, 2002, 73, pp.21–29.

84 C. Chiao, A.M. Carothers, D. Grunberger, G. Solomon, G.A. Preston and J.C. Barrett, Apoptosis and altered redox state induced by caffeic acid phenethyl ester (CAPE) in transformed rat fibroblast cells, *Cancer Res.*, 1995, 55, pp.3576–3583.

85 Y.T. Lee, M.J. Don, P.S. Hung, Y.C. Shen, Y.S. Lo, K.W. Chang, C.F. Chen, and L.K. Ho, Cytotoxicity of phenolic acid phenethyl esters on oral cancer cells, *Cancer Letters*, 2005, 223, pp.19–25.

86 U. Ketsawatsakul, Modulation by bicarbonate of the protective effects of phenolic antioxidants on peroxynitrite-mediated cell cytotoxicity, *Science Asia*, 2007, 33, pp.273-282.

87 O. Koru, F. Avcu, M. Tanyuksel, A.U. Ural, R.E. Araz and K. Şener, Cytotoxic effects of caffeic acid phenethyl ester (CAPE) on the human multiple myeloma cell line, *Turk. J. Med. Sci.*, 2009, 39, pp.863-870.

88 I. Basic, N. Orsolic, Z. Tadic, N.M.F. Alcici, A. Brbot-Saranovic, K. Bendelja, B. Krsnik and S. Rabatic, Antimetastatic effects of propolis, caffeic acid phenethyl ester and caffeic acid on mammary carcinoma of CBA mouse, 17th International Cancer Congress, 1998, pp. 63-75.

89 W. Ding, L.G. Hudson, X. Sun, C. Feng and K.J. Liu, As (III) inhibits ultraviolet radiation-induced cyclobutane pyrimidine dimer repair via generation of nitric oxide in human keratinocytes, *Free Radic. Biol. Med.*, 2008, 45, pp.1065-1072.

90 V. Staniforth, L.T. Chiu and N.S. Yang, Caffeic acid suppresses UVB radiation- induced expression of interleukin-10 and activation of mitogen- activated protein kinases in mouse, *Carcinogenesis*, 2006, 27, pp.1803-1811.

91 Y. Yang, Y. Li, K. Wang, Y. Wang and W. Yin, P38/NF-kB/Snail pathway is involved in caffeic acid-induced inhibition of cancer stem cells-like properties and migratory capacity in malignant human keratinocyte, *PLoS ONE*, 2013, 8.

92 M. Hirose, Y. Takesada, H. Tanaka, S. Tamano, T. Kato and T Shirai, Carcinogenicity of antioxidants BHA, caffeic acid, sesamol, 4- methoxyphenol and catechol at low doses, either alone or in combination, and modulation of their effects in a rat medium-term multi-organ carcinogenesis model, *Carcinogenesis*, 1998, 19, pp. 207–212.

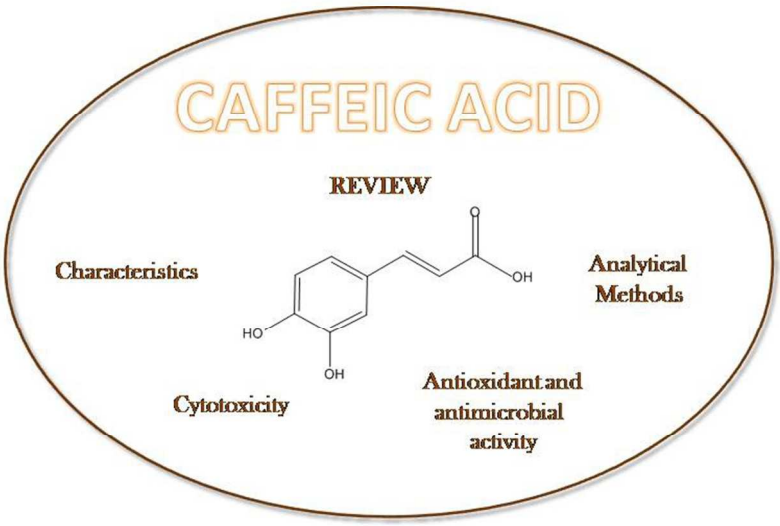
93 H. Wang, G.J. Provan and K. Helliwell, Determination of rosmarinic acid and caffeic acid in aromatic herbs by HPLC. *Food Chemistry*, 2004, 87, pp.307–311.

94 N.B. Cech, M.S. Eleazer, L.T. Shoffner, M.R. Crosswhite, A.C. Davis and A.M. Mortenson, High performance liquid chromatography/electrospray ionization mass spectrometry for simultaneous analysis of alkamides and caffeic acid derivatives from *Echinacea purpurea* extracts, *J. Chromatogr. A*, 2006, 1103, pp. 219–228.

95 L.L. Saldanha, W. Vilegas and A.L. Dokkedal, Characterization of flavonoids and phenolic acids in *Myrcia bella* Cambess using FIA-ESI- IT-MSn and HPLC-PAD-ESI-IT-MS combined with NMR., *Molecules*, 2013, 18, pp. 8402-8416.

96 P.A. Guy, M. Renouf, D. Barron, C. Cavin, F. Dionisi, S. Kochhar, S. Rezzi, G. Williamson and H. Steiling, Quantitative analysis of plasma caffeic and ferulic acid equivalents by liquid chromatography tandem mass spectrometry, *J. Chromatography B*, 2009, 877, pp. 3965–3974.

97 S.J. Wang, Z.Q. Zhang, Y.H. Zhao, J.X. Ruan and J.L. Li, Simultaneous quantification of chlorogenic acid and caffeic acid in rat plasma after an intravenous administration of mailuoning injection using liquid chromatography/mass spectrometry, *Rapid Commun. Mass Spectrom.*, 2006, 20, pp. 2303–2308.



254x190mm (96 x 96 DPI)