Tea (*Camellia sinensis* (L.)): a putative antimicrobial agent in sexually transmitted infections

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Much research is available depicting the health benefits of tea (*Camellia sinensis* (L.)) for a wide variety of implications, including Sexually Transmitted Infections (STIs). Acquired Immunodeficiency Syndrome (AIDS), Human Papilloma Virus (HPV) infections, vulvovaginal candidosis and vaginal bacteriosis are some of the most prevalent STIs. Several scientific studies indicate that tea is an excellent and inexpensive natural source of polyphenols, such as catechin derivatives, which are well known for their strong antimicrobial activities and are considered by many researchers as the main responsible for the beneficial health effects of this botanical species and its derivatives (extracts).

In this chapter, we pretend to emphasize the antimicrobial properties of this plant and of its major components. Thus, we will discuss briefly the aspects of its origin, cultivation and manufacturing process and will be focused in those related to its chemical composition and antimicrobial activity, especially against agents involved in the most prevalent STIs. The vaginal therapeutic delivery systems that vehicle tea extracts/components will also be included.

Keywords antimicrobial activity; *Camellia sinensis*; catechins; sexually transmitted infections

1. Introduction

Vagina is inhabited by a variety of microorganisms in a dynamic ecosystem in normal healthy women. Any disturbance of this microenvironment may lead to the development of several infectious conditions and diseases. Sexually transmitted infections (STIs), such as acquired immunodeficiency syndrome (AIDS), Human Papilloma virus (HPV) infections, vulvovaginal candidosis (VVC) and bacterial vaginosis (BV) are gaining significant importance at present due to their rapid dissemination, high cost of treatment, and the increased risk of transmission of other STIs [1].

The medicinal effects of tea (*Camellia sinensis* L.) have a long and rich history, dating back almost 5000 years. It is this long safety record of tea consumption that makes this botanical species and its phytochemicals attractive targets for drug discovery [2-5]. The chemical composition of tea includes hundreds of compounds, namely polyphenols, methyl-xanthines, carbohydrates, proteins, free amino acids, organic acids, volatile compounds, among many others [2-6].

In recent years, much attention has been focused on the role of tea flavonoids in the promotion of health, especially of catechins. In plants, these metabolites are involved in their protection against several pathogens including insects, bacteria, fungi, and viruses [7]. In the human organism, these polyphenols may exert health promoting properties, mainly antioxidant, anticancer, anti-inflammatory, antidiabetic and antimicrobial activities [2-6, 8, 9]. Recently, Bansal et al. (2013) described *C. sinensis* as a native source of antimicrobial agents. In fact, tea and its derivatives, which have been shown to possess effective activity against several sexually transmitted pathogens [10-12], are gaining popularity in the treatment of STIs. This recognition is related to their advantages when compared to conventional antimicrobial drugs such as less side effects, better patient tolerance, lower cost and acceptance due to long history of use. Tea, in general, and green tea, in particular, seem to be an excellent source of safe, effective and economical active agents and can be less prone to emergence of drug resistance than current antimicrobial drugs.

In this review, we will discuss briefly the aspects of tea origin, cultivation and manufacturing process and will focus on the most relevant reports about the antimicrobial activity of *C. sinensis* extracts (and its phytochemicals) upon microorganisms most frequently involved in STIs, such as *Candida* spp., *Gardnerella vaginalis*, HPV and Human immunodeficiency virus (HIV). The relationship between their composition and the antimicrobial effects will be highlighted and vaginal therapeutic delivery systems that vehicle tea extracts will be included.

2. Origin and cultivation

Tea plant, *C. sinensis*, a cultivated evergreen shrub of the Theaceae family, is native to China. Archeological research suggests that *C. sinensis* culture is likely to have begun in Southeast China more than 5000 years ago, from where it was brought to Japan and India, and further into many tropical/subtropical countries [13]. Since the end of 19th century, tea has also been produced in one single place in Europe - S. Miguel Island (Azores Archipelago, Portugal) [14, 15]. Today, tea is cultivated in more than thirty countries in the world [5, 16] but China remains the principal producer, followed by India, Kenia, Sri Lanka, Indonesia, Turkey, and Vietnam [2].

There are two major varieties of tea, botanically classified according to their leaf size: *C. sinensis* var. *sinensis* (China tea), the small-leaved variety, which grows extensively in China and Japan, and *C. sinensis* var. *assamica* (Assam tea), the large-leaved variety, which predominates in India. Much hybridization has occurred between Assam and China varieties [2].

3. Classification and manufacturing process

The infusion of the leaves of *C. sinensis* (frequently in the proportion of 1 g of dried leaves to 100 ml of boiling water) is also known as tea, being the most ancient and widely consumed beverage all over the world. In fact, tea has been used for centuries by ancestral cultures for its sensorial properties, relatively low retail price, stimulating effects and potential health benefits [14, 15, 17].

Commercial teas are usually classified into three major categories: unfermented (white and green teas), semifermented (oolong tea) and fully fermented (black tea) forms. In 2000, approximately 78% of the total amount of tea produced and consumed worldwide corresponded to black tea, 20% to green tea, and less than 2% to white and oolong teas [16]. According to Katiyar and Mukhtar (1996), tea presents an average per capita intake of around 120 ml/day, being the second most widely consumed drink worldwide (next to water) [18].

Green, oolong, and black teas are all obtained from the leaves of *C. sinensis*, differing in their appearance, taste, flavor and chemical composition according to the extent of the fermentation process [2-5]. After harvesting, tea leaves began to wilt very quickly and enzymatic oxidation begins, if they are not dried very quickly. During oxidation process, they turn progressively darker since chlorophylls break down and tannins are released. In tea industry, enzymatic oxidation (generally followed by polymerization) is commonly called “fermentation”, and may be blocked at a predetermined stage by heating. This thermal processing inhibits the enzyme polyphenol oxidase responsible for this phenomenon.

In the production of green teas, freshly harvested leaves are rapidly steamed or pan-fried in order to inactivate polyphenol oxidase enzyme, thereby preventing oxidation of catechins and producing a dry and stable final product [2, 5, 16]. Its composition is very similar to that of *C. sinensis* leaves.

Oolong and black teas are produced from fresh leaves, which are allowed to wither until their moisture content is reduced to about 55% of the original leaf weight, resulting in catechins concentration [16]. The withered leaves are then rolled and crushed, promoting polyphenols oxidation and polymerization [2, 5, 16]. The main difference between oolong and black teas is in the degree of oxidation/polymerization of catechins, which is lower in oolong tea.

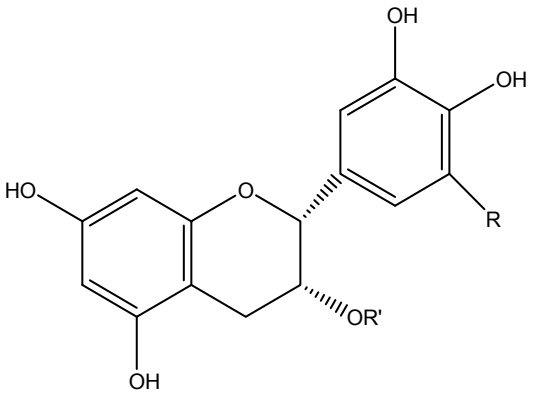
White tea is very similar to green tea but it is exceptionally prepared only from the buds and young tea leaves, while as previously referred green tea is prepared from the maturated leaves. The delicate buds and young leaves are rapidly steamed, in order to prevent withering and enzymatic oxidation. Therefore, this collection and manufacturing processes provide some of the most exceptional, subtle and expensive teas [6]. White tea composition is very similar to that of *C. sinensis* buds/young leaves.

4. Chemical composition

The phytochemical components of tea leaves include polyphenols (e.g. catechins and their derivatives), methyl-xanthines (e.g. caffeine, theophylline and theobromine), carbohydrates, proteins, free amino acids, vitamins (e.g. vitamin C and carotenoids), volatile compounds, lipids, chlorophylls, saponins and inorganic elements (e.g. fluorine, manganese and aluminium) [2-4, 19].

Phenolic compounds seem to be the most important group of tea leaves components, due to their higher relative abundance (up to 30% of the dry weight of the water-extractable material) and bioactive properties [2, 5, 11, 15-17, 20-28]. In fact, a green tea infusion (200 mL) might contain up to 200 mg of polyphenols [29]. Generally, the total phenolic content of white tea is similar or, sometimes, even higher than that found in green tea [6, 30]. However, the number of investigations to study white tea is insignificant when compared to the volume of papers on green tea.

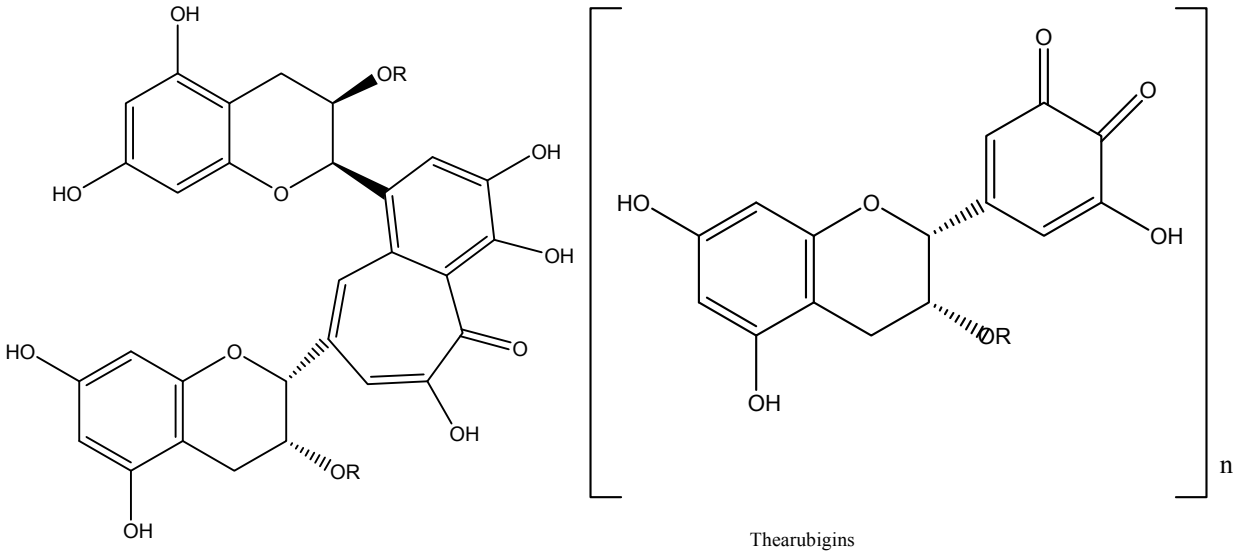
Most of the polyphenols in white and green teas are flavan-3-ols, also called catechins. The four major catechins (Fig. 1) are (-)-epicatechin, (-)-epigallocatechin, (-)-epicatechin-3-gallate, and (-)-epigallocatechin-3-gallate [2, 16, 21]. This last compound is the predominant and its health promoting abilities have been extensively studied by several authors [5, 20, 24, 25, 28, 31-37].



R=H, R'=H, (-)-epicatechin
R=OH, R'=H, (-)-epigallocatechin
R=H, R'=3,4,5-Trihydroxybenzoyl, (-)-epicatechin-3-gallate
R=OH, R'=3,4,5-Trihydroxybenzoyl, (-)-epigallocatechin-3-gallate

Fig. 1 Chemical structures of the main tea catechins.

Postharvest inactivation of polyphenol oxidases in green tea leaves and white tea buds/young leaves prevents oxidation of their catechins, whereas enzyme-catalized oxidation and polymerization of catechins result in the formation of theaflavins (dimers) and thearubigins (polymers) (Fig. 2), which are less soluble and confer a characteristic colour and flavour to oolong and black teas [5, 7, 38]. Fermentation step significantly reduces the total content of flavan-3-ols [2, 4, 6]. When compared to black tea, oolong tea is semifermented and presents a mixture of catechins, theaflavins and thearubigins [5].



R=H, Theaflavin
R=3,4,5-Trihydroxybenzoyl,theaflavin-3,3'-digallate

Thearubigins

Fig. 2 Chemical structures of the main theaflavins and thearubigins.

Phenolic acids (gallic, 5-*O*-galloylquinic, *p*-coumaroylquinic, 3-*O*- and 5-*O*-caffeoylquinic acids), flavonols (quercetin, kaempferol and myricetin and their glycosides) and flavones (vitexin and isovitexin) are also commonly found in white, green, oolong and black teas [14, 15, 17, 38].

Besides polyphenols, tea contains other compounds with considerable interest for human health such as caffeine, theophylline, theobromine and L-theanine [2]. The stimulant effects of tea on the central nervous, cardiovascular and renal systems have been attributed to the presence of the methylxanthines - caffeine, theophylline, and theobromine [39, 40]**.** Caffeine is the predominant methylxanthine, comprising about 3-6% of the water-extractable material [2, 21]. The leaves of *C. sinensis* contain about 17% of nitrogen compounds, in the form of proteins, free amino acids, and nucleic acids [41]. Among free amino acids, L-theanine is the major one, corresponding to 60% of the total content [24, 42]. This amino acid is considered as the chemical marker of tea since it is exclusive of this botanical species [24, 42].

Besides collection and manufacturing processes, other factors such as variety, geographical origin, climate, soil composition or harvesting season also influence the composition of tea [2, 5, 16]. In addition to this variability is the susceptibility of tea compounds to extraction by using different extraction conditions: solvents (water, hydroalcoholic mixtures, methanol, ethanol), temperatures and times of extraction [7, 43, 44]. All these factors need to be considered in order to maximize extraction of the antimicrobial tea components.

5. Antimicrobial activity against genital microorganisms

Vaginal administration of natural products to control and eradicate genital infections is very popular among women and arises as a possible alternative to overcome antibiotic resistance [1, 45]. In fact, natural products seem to be valuable for topical therapy especially in recurrent and resistant cases. In addition to the limited number of available antimicrobials, the restrictions to its use and the increasing number of resistant cases stress the need for the development and validation of new therapeutic strategies exhibiting distinct mechanisms of action and/or evasion of resistance [46].

5.1. HIV

During the last decades HIV became one of the major STI. Several efforts have been made to find effective ways to control its dissemination by sexual route being the development of effective, safe and affordable therapies pursued. Until now the major compounds approved by FDA for HIV treatment are HIV reverse transcriptase inhibitors, protease inhibitors and few HIV entry inhibitors [47]. Due to the emergence of drug-resistant HIV mutants and adverse effects related to these anti-viral drugs, the development of new classes of anti-HIV drugs with new targets, such as the virus entry, integration and maturation are needed. Tea extracts, specifically, green and black tea extracts rich in catechins and theaflavins respectively, have been shown to exhibit anti-HIV properties. EGCG, has been reported to inhibit HIV-1 replication, as it interferes with proteins activity, specifically reverse transcriptase and protease, inactivating virions [48-50]. Additionally, both catechins and theaflavins were found to be able to inhibit virus entry in host cell by blocking HIV-1 envelope glycoproteins interaction with cell membrane [51, 52]. Theaflavins were considered more potent than catechins [51]. Pre-clinical assay evidenced EGCG efficacy to inhibit HIV-1 infectivity on human CD4 T cells and macrophages, in a dose dependent manner [12].

5.2. HPV

HPV infection is a very common genital infection [53]. This infection is usually self-controlled but it can persist, being HPV related to cervical cancer development [54]. Local therapy is widely used in HPV infections as systemic therapy is highly ineffective [55].

Polyphenon E (Veregen®), an ointment consisting of a high grade decaffeinated green tea water extract (more than 85% of *C. sinsensis* catechins), represents the first botanical product approved by the United States FDA for the treatment of a human disease [32]. This plant-based medicine is used in the topical treatment of anogenital warts, which result from infection caused by certain strains of HPV. Green tea catechins effectiveness in eradicating these warts results from antiviral, immunostimulatory, and antioxidant mechanisms, with negligible toxicity to humans [32, 56-58].

The *in vitro* antiproliferative activity of EGCG against cervical cancer cells has also been reported [20, 56]. This catechin inhibits cervical cancer cell growth through induction of apoptosis and cell arrest, and regulation of gene expression. In addition, clinical efficacy of green tea extracts and catechins delivered in the form of ointments and/or capsules to patients with HPV infected cervical lesions was demonstrated by the same group [11].

5.3. *Candida* spp

Candida spp are microorganisms frequently found in the human oral cavity, gastrointestinal tract and vagina [59-62]. Concerning mucocutaneous infections, VVC is the second most frequent vaginal infection, after BV. In fact, VVC is the most common clinical disease caused by *Candida* spp., affecting 70-75% of women at least once in their lifetime [63-65]. Due to the growing number of resistant clinical cases and the reduced diversity of antifungal compounds, new therapeutic strategies have been studied [46]. Tea extracts have been considered a putative valuable source of active molecules against Candida. The antimycotic activity towards *C. albicans* was higher in non-fermented (white and green teas) than in semi-fermented (red tea) or fermented (black tea) teas [10]. Tea catechins antifungal effects upon *C. albicans*, were previously described, alone or combined with classic antifungals, such as amphotericin B and fluconazole [66]. Pyrogallolcatechins (EGCG, epigallocatechin, gallocatechin and gallocatechin-3-gallate) exhibited stronger antifungal activity against *C. albicans* than catechol catechins (epicatechin, epicatechin-3-gallate, catechin and catechin-3-gallate). The anti-Candida effect of EGCG, epigallocatechin and gallocatechin was found fungicidal, pH dependent and synergic when combined with classic antifungals [66]. A disturbance in *C. albicans* folic acid metabolism and consequently an inhibition of ergosterol synthesis was found to be related with ECGC mechanism of action toward yeasts. According to these researchers, this effect could explain the synergic effect between EGCG and azole antimycotics [67].

Moreover, the anti-Candida effect of green tea was also described against non-albican species [68, 69]. Green tea exhibited activity towards *C. glabrata* (MIC of 1.2 mg/mL corresponding to 0.5 mg of green tea polyphenols/mL) and both epicatechin-3-gallate and EGCG have shown activity against *C. glabrata* resistant to ketoconazole [68]. *C. glabrata* was shown to be the most susceptible species, followed by *C. guilliermondii*, *C. parapsilosis* and *C. krusei* [69].

5.4. *Gardnerella vaginalis*

Among genital infections, BV is considered the most prevalent vaginal infection in women between 15 and 44 years old [70-72]. Despite BV being a polymicrobial infection, *Gardnerella vaginalis* is present in 95% of clinical cases, playing an important role in this infection etiology [73-76]. Therapeutic options for VB treatment are very scarce and microorganisms resistance is becoming an important constraint [77, 78]. Despite bibliography being not extensive concerning reports on tea extracts anti-*Gardnerella* activity some data is available. In fact, a new product for topical application that vehicles polyphenon E extracted from green tea, was proposed for the treatment of BV [79].

6. Other biological activities

The chemical constituents in tea contribute to important biological activities of this plant, playing an important role on human health. Several *in vitro* and *in vivo* studies, as well as clinical and epidemiologic studies concerning different types of teas, like black, green or white, support these potential health benefits. The tea polyphenols in general and EGCG in particular, have been described as the responsible for tea medicinal potentialities.

The extracts of *Camellia sinensis* have been described as possessing important antioxidant activity that are correlated with the high polyphenol content [80-83]. These antioxidant properties are beneficial for several chronic diseases related with oxidative stress, including cancer, cardiovascular and neurodegenerative diseases.

The preventive and inhibitory activities of *Camellia sinensis* and its chemical constituents against carcinogenesis at different organs have been demonstrated in many animal models and cell culture systems [21, 84-87]. On the other hand, although several studies suggest a reduction of cancer risk in humans induced by tea consumption, especially green tea [88-90], the results have not been consistent [88, 91].

Green tea polyphenols have been reported as being beneficial for the cardiovascular system, probably by lowering cholesterol and preventing platelet aggregation, as well as by inhibiting lipid peroxidation [3, 84]. Epidemiological studies also suggested a significant association between black tea consumption and a reduced risk of coronary disease [92].

Animal studies, as well as epidemiological ones, have demonstrated that aqueous extracts of *Camellia sinensis* (black, green and white tea) are effective to prevent development of diabetes, Type 1 or type 2 [3, 30, 92]. There is also strong evidence that green tea improves weight loss and maintenance, through fat oxidation [93].

Human epidemiological data and animal studies suggested that the pharmacological benefits of tea may help to protect the brain against the ageing process. It has been reported that tea consumption is inversely correlated with the incidence of neurodegenerative diseases as Alzheimer's and Parkinson's diseases [3, 84].

Several other health benefits have been described for *Camellia sinensis* aqueous extracts, however when the tea consumption exceeds certain limits, some detrimental effects on human health could be observed. Side effects like hepatotoxicity, reduced iron absorption, oxidative stress or precipitation of digestive enzymes can be observed [94].

As noted above, the chemical constituents in tea are pharmacologically active and some of them might interact with synthetic drugs, acting by synergy or inhibition. Green tea should be used with caution in patients taking analgesics, antivirals, cytochrome P450-metabolized agents and hormonal agents, among others [95].

7. Vaginal delivery systems with tea extracts

Generally the therapeutic use of plant extracts has been achieved by incorporation in different dosage forms such as capsules or pills, infusions, creams, ointments, gels, patches, syrups and elixirs [96]. Traditional vaginal dosage forms range from tablets, capsules and suppositories to liquid (solutions) and semi-solid formulations such as gels and creams [97].

Veregen®, a genital ointment based on Polyphenon E represents the first botanical product approved by the United States Food and Drug Administration (FDA) for the treatment of a human disease. Polyphenon E is a proprietary high grade decaffeinated aqueous extract of green tea leaves that contains more than 85% of *C. sinsensis* catechins [32]. This plant-based medicine is used in the topical treatment of anogenital warts, which result from infection caused by certain strains of HPV and has been proved to be effective, with low recurrence rates while rather safe and tolerable [98]. Green tea catechins effectiveness in eradicating these warts results from antiviral, immunostimulatory, and antioxidant mechanisms, with negligible toxicity to humans [32, 56-58]. Veregen® was approved in 31th October 2006 and its patent is protected until 2017 [32]. It represents the only genital delivery system based on tea extracts for therapeutic use currently approved by FDA.

Herbal compositions containing green tea among other plant extracts have been patented as suppositories to be used in vaginal infections [99] and vaginal tablets to prevent and treat cervical intraepithelial neoplasia [100].

The fact that most of the marketed vaginal formulations containing plant extracts are over-the-counter products may be associated with the scarcity of information on other formulations containing tea extracts.

Successful genital delivery of tea extracts for therapeutic purposes strongly depends on the development of formulations that may guarantee drug stability over storage and adequate therapeutic concentrations at the site of infection. Green tea catechins stability has been shown to be affected by neutral or alkaline pH, temperature, high oxygen concentration and metal ions. On the other hand, their low bioavailability represents a strong limitation for oral therapeutic applications [101] and it can also affect the effectiveness of topical treatment. Formulation factors and technologies may overcome these limitations. The development of advanced drug delivery systems such as microparticles, liposomes and nanoparticles has been shown to be a promising strategy for green tea cathechins delivery [101]. For instances, prevention of enzymatic changes and improvement of catechins ability to permeate skin has been achieved for green tea extract-loaded chitosan microparticles [102]. The development of new targeted tea extracts-based vaginal delivery systems may, therefore, rely on the adequate selection of excipients and formulations.

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