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Camellia annamensis (Theaceae): phytochemical analysis, cytotoxic, antioxidative, and antimicrobial activities

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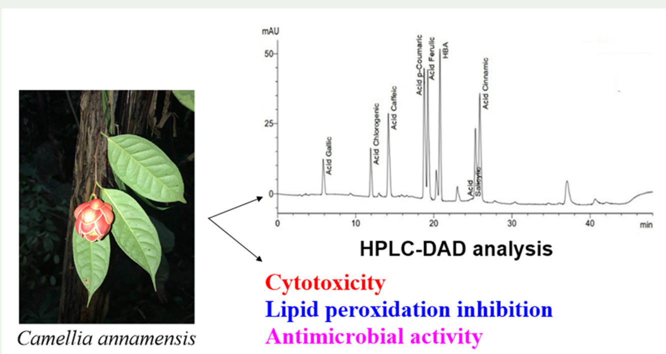
ABSTRACT

Cytotoxic, antioxidative, and antimicrobial activities of *Camellia annamensis*, and its chemical compositions were first provided in the current study. Phenolic contents in the methanol extracts of its leaves and flowers were 222.73±0.09 and 64.44±0.08 mg GAE/g extract, whereas flavonoid contents in these parts were 108.80±0.28 and 131.26±0.39 mg rutin/g extract, respectively. By using HPLC-DAD analysis, gallic acid (43.72±0.09−81.89±1.83 mg/g) and (–)-epigallocatechin gallate (67.31±1.26−70.68±7.82 mg/g) were identified as the major compounds. *C. annamensis* leaf and flower extracts were moderately cytotoxic against A549, HT-29, SK-Mel-2, MCF-7, HepG2, HeLa, and MKN-7. Particularly, they are better than the standards trolox (IC_{50} 7.57±0.23 µg/mL) in lipid peroxidation inhibitory evaluation, and streptomycin (IC_{50}/MIC = 45.34–50.34/128–256 µg/mL) in antimicrobial assay against the Gram-positive bacteria *Enterococcus faecalis* ATCC299212, *Staphylococcus aureus* ATCC25923, and the Gram-negative bacterium *Salmonella enterica* ATCC13076.



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1. Introduction

Cancer, the leading cause of mortality worldwide and the cause of 9.6 million deaths in 2018, has been one of the health issues that is by far the most alarming (Bray et al. 2018). 12 million fatalities predicted by 2030 are a remarkable number that is obviously viewed to drastically climb (Wu et al. 2023). Over 100 different types of cancer are recognised. Breast cancer is the most prevalent types of cancer in the world at present, followed by prostate, lung, ovarian, colon, or rectal cancers (Mohibi et al. 2011). Breast cancer is the second-leading cause of cancer-related deaths in women globally, resulting in over 50% of all breast carcinomas being oestrogen receptor-positive (Mohibi et al. 2011). As is evident, it is challenging to prevent the occurrence of negative side effects from chemotherapy medications during treatment. Since natural products have impressively antioxidative properties supporting their uses as adjuvants to anticancer treatments to minimise the consequences of oxidative damage, numerous attempts have been made to find natural agents from medicinal plants in the interim (Ozkur et al. 2022; Huan et al. 2023).

As we know, the liver performs a variety of essentially biological tasks, including protein synthesis, biochemical digestion, and detoxification (Tajiri and Shimizu 2013). Most of the detoxifying progress has been made by oxidative processes, which have boosted ROS (reactive oxygen species) production (Thuy et al. 2023). Through the medium of lipid peroxidation, the excessive creation of ROS results in DNA damage, protein adduct formation, mitochondrial dysfunction, and liver disease (Elshamy et al. 2020). Lipid peroxidation is the process where unsaturated fatty acids react with oxygen. This reaction is possibly catalysed by enzymes like lipoxygenases or cyclooxygenases (Schneider 2009). Products of lipid peroxidation include lipid hydroperoxides, and 4-hydroxynonenals, especially malondialdehyde (MDA) (Fedorova et al. 2019). By modifying lipid-lipid interactions, membrane fluidity, and permeability, these chemicals primarily cause cellular damage (Fedorova et al. 2019). However, antioxidants may be able to counteract this process.

The development of new antimicrobial compounds that can combat drug resistance was prompted by the pathogenic bacteria's increasing resistance to antibiotics, and the ongoing emphasis on reducing healthcare costs. The use of Vietnamese tropical plants as antibacterial components in herbal medication formulations has long been acknowledged. As an example, the growth of seven Gram-positive strains, including *Bacillus cereus*, *Escherichia coli*, *Staphylococcus aureus*, *S. epidermidis*, *Streptococcus pneumonia*, and *Streptococcus pyogenes*, was inhibited by methanol extracts of *Dalbergia tonkinenis* leaves, stem barks, and roots at a concentration of 1.0 mg/mL (Ninh et al. 2018). With a low minimum inhibitory concentration (MIC) of 100 µg/mL, the petroleum ether extract of other Vietnamese medicinal plant *Jasminum subtriplinerve* leaves and stems proved effective against the Gram-positive bacterium *B. subtitis* (Ngan et al. 2008). Therefore, the search for antimicrobial agents from medicinal plants is likely to be a good strategy.

Tea can be seen as the largest consumed beverage in the world and is often obtained from *Camellia* leaves. More than 220 *Camellia* species have been recorded to date, which are mostly distributed in tropical Asia regions (An et al. 2023). Among them, *C. sinensis* leaves and flowers are popular commercial goods due to their remarkable health benefits, such as anticancer, antioxidant, anti-inflammatory, anti-hepatotoxic, and tyrosinase-inhibiting activities (Sharangi 2009). Many experimental attempts have been performed to study the chemical identifications and pharmacological values of *Camellia* species, in which phenolics were the principal compounds (Teixeira and Sousa 2021). *Camellia* extracts exert versatile pharmacological features with antioxidative activity being the most valuable (Zhang et al. 2019; Teixeira and Sousa 2021). Recently, *Camellia annamensis* N.S.Lý, V.D.Lương, N.Đ.Đỗ, T.H.Lê & T.L.Nguyễn was described as a new species available in central Vietnam (Ly et al. 2022). The ultimate goal of this study is to offer new information on phytochemical analysis, cytotoxicity, lipid peroxidation inhibition, and antimicrobial activity of the methanol extracts of *C. annamensis* leaves and flowers. The results might be used to guide further studies on this species.

2. Results and discussion

2.1. Phytochemical analysis

The extraction yields of *C. annamensis* leaves (CAL) and flowers (CAF) are 10.75 g/1.0 kg dried powder, 3.50 g/1.0 kg dried powder, respectively. The initial qualitative screenings clearly show that both extract samples contain phenolics type flavonoids, and tannins (Figures S1, S2). Phenolic contents of the leaves and flowers represent 222.73 ± 0.09 mg GAE/g extract and 64.44 ± 0.08 mg GAE/g extract, respectively (Table S1). In the meantime, flavonoid contents in the leaves and flowers are equivalent to 108.80 ± 0.28 mg rutin/g extract and 131.26 ± 0.39 mg rutin/g extract, respectively (Table S1). It suggests that *Camellia* extracts are promising resources of phenolic compounds. As an example, the total phenolic contents in the methanol extracts of Chinese *C. ptilophylla* and *C. kucha* young shoots corresponded to 280.74 and 234.08 mg GAE/g extract, whereas this value assigned to methanol extract of Korean *C. sinensis* was 223 mg GAE/g extract (Ku et al. 2010; Li et al. 2012). Meanwhile, the total of flavonoid content of the ethanol extract of Chinese *C. japonica* leaves was 205.19 mg rutin/g (Pereira et al. 2023). In another report, the flavonoid amount in Chinese *C. oleifer*a fruit reached up to 140.06 mg quercetin/g (Zhang et al. 2011).

The qualitative and quantitative actions were also carried out using the HPLC-DAD tool at λ 295 and 340 nm. Based on comparisons with the UV spectral data and retention time (Rt) of phenolic standards, compounds in the methanol extracts of *C. annamensis* leaves and flowers were identified (Table S2 and Figure S3). Eleven phenolics, including gallic acid, chlorogenic acid, caffeic acid, *p*-coumaric acid, ferulic acid, HBA (2,4-dihydroxybenzoic acid), salicylic acid, cinnamic acid, rutin, quercetin, and EGCG ((–)-epigallocatechin gallate) were elucidated, in which LOD (limits of detection) and LOQ (limit of quantitation) ranged from 0.01 to 2.46 µg/mL, and 0.05 to 8.20 µg/mL, respectively. As shown in Table S2, HBA (17.12 ± 0.21 mg/g dried leaves and 19.78 ± 0.06 mg/g dried flowers), gallic acid (43.72 ± 0.09 mg/g dried leaves and 81.89 ± 1.83 mg/g dried flowers), and EGCG (67.31 ± 1.26 mg/g dried leaves and 70.68 ± 7.82 mg/g dried flowers) are the principal compounds. Caffeic acid and salicylic acid were reported to achieve about 5–10 mg/g. Accumulative evidence reveals that gallic acid and EGCG are likely to be the main phenolic compounds of other *Camellia* species. The contents of gallic acid in the dried fruit shells of Chinese *C. polyodonta*, *C. oleifera*, *C. oleifera* var *monosperma*, *C. pitardii*, and *C. grijsii* were 58.15, 29.72, 7.50, 9.29, and 7.43 mg/kg (Shen et al. 2022). EGCG accounted for 115 mg/g in Australian fresh shoots of green tea (Yao et al. 2004). In other examples, it was found to reach the highest amounts in Chinese teas, including 3.6, 4.58, 2.29, and 7.28% (w/w dried leaves) in *C. sinensis*, *C. assamica*, *C. ptilophylla*, and *C. assamica* var. *kucha*, respectively (Peng et al. 2008).

2.2. Cytotoxic activity

Two methanol CAL and CAF extracts were first subjected to cytotoxic examination against a panel of seven cancer cell lines A549, HT-29, SK-Mel-2, MCF-7, HepG2, HeLa, and MKN-7 (Table S3). In general, *C. annamensis* extracts moderately control the growth of these cancers. At the highest tested dose of 100 µg/mL, the CAL and CAF extracts cause the inhibitory percentages of 52.56 – 82.85% and 55.29 – 72.64%, respectively. In comparison with the positive control ellipticine (IC_{50} 0.31–0.44 µg/mL), the CAL and CAF extracts possess the IC_{50} values of 33.78–94.80 µg/mL and 49.06–84.56 µg/mL, respectively. It was in agreement with previous reports since various pieces of evidence showed that *Camellia* extracts themselves were appropriate for cancer treatments. For example, the optimised pu-erh tea (*Camellia sinensis* var. *assamica*) extract, containing the main compounds gallic acid, caffeine, and theobromine, was cytotoxic against cancer cell lines Caco-2, A549 and HepG2 (Armstrong et al. 2022). Green tea with the high amount of phenolic EGCG was found to successfully induce p53-stimulated cytotoxicity, and inhibited migration of breast cancer MCF-7 and MDA-MB-231 cells (Santos et al. 2021). Cytotoxic activity of the water fraction of *C. japonica* leaves against cancer cell lines A549, SW480 and RPMI 1640 may be attributed to the presence of phenolic compounds, especially epicatechin and quercetin (Kim et al. 2010).

2.3. Antioxidative activity

Antioxidative effects of the methanol CAL and CAF extracts were evaluated based on their lipid peroxidation inhibitory ability. From Table S4, both these two extracts show better inhibitory percentages in each tested concentration than those of the positive control trolox. At a concentration of more than 20 µg/mL, *C. annamensis* extracts are associated with an inhibitory percentage of more than 80%, which are comparable with those of trolox (79.90–81.11%). As a consequence, the IC_{50} values of CAL (1.56 ± 0.07 µg/mL) and (0.87 ± 0.05 µg/mL) are much better than that of trolox (IC_{50} 7.57 ± 0.23 µg/mL). Apparently, the obtained results were dependent on the high amounts of polyphenols and flavonoids, as well as the role of well-known phenolic compounds, like gallic acid, HBA, and especially EGCG. In the same model, the alcoholic extracts of two other Vietnamese *Camellia* plants *C. vuquangensis* and *C. hatinhensis* possessed the IC_{50} values of 7.92–17.45 µg/mL (An et al. 2023). It turns out that Japanese dietary green and black tea have lipid peroxidation inhibitory potentials in rat's liver and kidney *ex vivo* (Sano et al. 1995). It was found that the rich polysaccharide fraction of tea (74 and 294 µg/mL) exhibited microsomal lipid peroxidation prevention (Chen et al. 2008). Our current results, once again, confirmed the great value of *Camellia* constituents in antioxidative treatments.

2.4. Antimicrobial activity

Both the CAL and CAF samples were further evaluated for antimicrobial activity against three Gram-positive bacterial strains *E. faecalis*, *S. aureus*, and *B. cereus*, three Gram-negative bacterial strains *E. coli*, *P. aeruginosa*, and *S. enterica*, and one yeast strain *C. albicans*. From Table S5, two tested samples with the IC_{50}/MIC = 9.36–43.23 µg/mL/32–128 µg/mL are better than the standard streptomycin (IC_{50}/MIC = 45.24–50.34 µg/mL/256 µg/mL) against the Gram-positive bacteria *E. faecalis* and *S. aureus*. Two samples also show a strong inhibition of the growth of *B. cereus* with the IC_{50}/MIC = 43.25–89.32 µg/mL/128–256 µg/mL. In the same manner, these alcoholic extracts are strong against the Gram-negative bacteria *E. coli* (IC_{50}/MIC = 10.32–20.43 µg/mL/64 µg/mL) and *S. enterica* (IC_{50}/MIC = 19.35–38.46 µg/mL/64–128 µg/mL). However, only CAL sample possess activity against the Gram-negative *P. aeruginosa* (IC_{50}/MIC = 98.47 µg/mL/256 µg/mL). Lastly, these extracts were observed to strongly suppress the growth of fungus *C. albicans* with the IC_{50}/MIC = 12.38–14.02 µg/mL/64–128 µg/mL, when cycloheximide was used as a positive control (IC_{50}/MIC = 10.46 µg/mL/32 µg/mL). Feás et al. (2013) suggested that the seed oils of *C. oleifera*, *C. reticulate*, and *C. sasanqua* were better than the positive control gentamicin in an antimicrobial assay against *E. coli*, and they showed moderate activity against *B. cereus*, and *C. albicans*. It turns out that the methanol extract of *C. sinensis* leaves was found to be the most effective against *B. cereus* (Chakrabort and Chakrabort 2010). Hence, it can be safe to deal with the application of *Camellia* extracts to treat bacterial infections.

3. Experimental

See supplementary material.

4. Conclusion

The methanol extracts of *C. annamensis* leaves and flowers were associated with the predominance of phenolic acids and flavonoids. Two well-known compounds gallic acid and EGCG presented as the major metabolites. The obtained extracts moderately inhibited the growth of cancer cell lines A549, HT-29, SK-Mel-2, MCF-7, HepG2, HeLa, and MKN-7. In particular, these extracts are promising agents for antioxidative and antimicrobial treatments since their effects were comparable to the standards. Although the current study provides many promising examples, further detailed investigations and mechanisms of action are still needed to acknowledge the benefits of *Camellia* phenolics as food supplements.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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