

# Anticancer activity of the supercritical extract of Brazilian green propolis and its active component, artemillin C: Bioinformatics and experimental analyses of its mechanisms of action

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**Abstract.** Propolis, a resinous substance collected by honeybees by mixing their saliva with plant sources, including tree bark and leaves and then mixed with secreted beeswax, possesses a variety of bioactivities. Whereas caffeic acid phenethyl ester (CAPE) has been recognized as a major bioactive ingredient in New Zealand propolis, Brazilian green propolis, on the other hand, possesses artemillin C (ARC). In this study, we report that, similar to CAPE, ARC docks into and abrogates mortalin-p53 complexes, causing the activation of p53 and the growth arrest of cancer cells. Cell viability assays using ARC and green propolis-supercritical extract (GPSE) revealed higher cytotoxicity in the latter, supported by nuclear translocation and the activation of p53. Furthermore, *in vivo* tumor

suppression assays using nude mice, we found that GPSE and its conjugate with  $\gamma$  cyclodextrin ( $\gamma$ CD) possessed more potent anticancer activity than purified ARC. GPSE- $\gamma$ CD may thus be recommended as a natural, effective and economic anticancer amalgam.

## Introduction

Propolis is a complex mixture of resinous material, produced by bees, created by mixing their saliva with the botanical sources they live on. The color (yellowish green to dark brown) and odor (odorless to aromatic) of propolis vary and depend on its botanical source, origin of place and bee characteristics, such as strain and age (1,2). Apart from the structural and functional attributes of propolis for beehives (1), propolis has been reported to possess a variety of disease-preventive and therapeutic potentials for the human population (3). There are mainly two types of propolis known that differ in their constituents: New Zealand propolis that possesses caffeic acid phenethyl ester (CAPE) and Brazilian green propolis that possesses artemillin C (ARC) as predominant bioactive ingredients. Besides these, over 150 constituents, including flavonoids, phenolic acids, esters, terpenoids, steroids, amino acids and cinnamic acid derivatives have been identified (3), and are hence considered as popular pharmacological research material (4). A broad spectrum of biological activities identified in propolis include antitumor (5-12), anti-inflammatory (13-15), anti-bacterial (16-18), anti-viral (16,19,20) and anti-fungal (16) activities. Propolis is used in cosmetic products, such as body lotions, ointments, face creams and in functional food in various forms, such as tablets, capsules, toothpaste and mouthwash preparations (21). Molecular studies on the anticancer activity of propolis have revealed that its phenolic components cause cell cycle arrest and apoptosis (11), mitochondrial stress (22) and the inhibition of tumor growth (11,22,23). CAPE-based propolis extract (Bio-30) has been reported to block p21 (RAC1) activated

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**Abbreviations:** ARC, artemillin C; GPSE, green propolis supercritical extract;  $\gamma$ CD,  $\gamma$  cyclodextrin; DMEM, Dulbecco's modified Eagle's medium; CAPE, caffeic acid phenethyl ester; GADD45, growth arrest and DNA damage-inducible 45; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; BSA, bovine serum albumin; PBS, phosphate-buffered saline

**Key words:** Brazilian green propolis, supercritical extract,  $\gamma$  cyclodextrin conjugate, p53-mortalin complex, abrogation, anticancer

kinase 1 (PAK1) signalling and to suppress tumors in neurofibromatosis (9,24,25).

ARC (3,5-diprenyl-4-hydroxycinnamic acid) is one of the active phenolic acid components of Brazilian propolis generated by bees from materials from a Brazilian plant, *Baccharis dracunculifolia*. It has been shown to possess various biological activities, such as anti-viral (14), anti-bacterial (14,26), antioxidant (26) and anti-carcinogenic (14,26-28) activities. ARC has been reported to inhibit the growth of transplanted solid human and mouse tumors, including malignant melanoma in athymic and thymic mice, respectively (27). CAPE and ARC have been reported to differ in their bioavailability profile that in turn is determined by the stability of the compounds to the digestive enzymes and absorption through the intestinal lining. CAPE alone becomes degraded by secreted esterases (12); however, when combined with  $\gamma$  cyclodextrin ( $\gamma$ CD) it is protected and has improved activity in *in vitro* and *in vivo* antitumor assays (12). On the other hand, ARC has been reported to possess extremely low absorption efficiency and bioavailability. Whereas CAPE is absorbed and distributed by the monocarboxylic acid transporter (MCT)-mediated transport system (29), ARC is mainly permeated across by transcellular passive diffusion (30). We previously performed a cDNA array of CAPE-treated normal human cells and found that the cytotoxicity of CAPE was mediated by the activation of p53-growth arrest and DNA damage-inducible 45 (GADD45). Bioinformatics and experimental analyses revealed that CAPE targeted mortalin-p53 interactions, resulting in the nuclear translocation and re-activation of p53, leading to growth arrest in cancer cells (12). In the present study, we report that ARC possesses similar capabilities; however its cytotoxic efficacy is low. In order to increase the potential of ARC, we prepared the following: i) supercritical extract [green propolis supercritical extract (GPSE)]; and ii) its complex with  $\gamma$ CD (GPSE- $\gamma$ CD). We then tested their cytotoxicity in human cancer cells. We report that GPSE contains 9.6% ARC and exerted cytotoxic effects at 0.5% (16.6  $\mu$ M) and was equivalent to  $\sim$ 500  $\mu$ M of pure ARC in *in vitro* toxicity assays. The extract exhibited marked antimigratory activity. Furthermore, the *in vivo* efficacy of GPSE was significantly enhanced by its complex with  $\gamma$ CD.

## Materials and methods

**Docking of mortalin and p53 with ARC.** The crystal structures of human mortalin (PDB ID: 4KBO) and p53 (PDB ID: 1OLG) were obtained from Protein Data Bank (PDB) (31), while the ligand, ARC (Compound ID: 5472440) was from PubChem Compound (32) database. ArgusLab 4.0.1 (M 2004), a freely available molecular modelling, graphics and drug design package, was used for docking analyses. The structures of protein and the ligand molecules were prepared and the receptor grid around the binding site residues selected from the receptor was defined. The grid resolution was kept as 0.4 Å and an exhaustive search docking was performed. The docking process was carried out using the 'ArgusDock' docking engine, calculation type as 'Dock' and the ligand was kept in rigid mode. The scoring function used was an empirical scoring function, 'AScore', which takes into account van der Waals energy, hydrophobic component, hydrogen bond and deformation penalty. The parameter file,

'AScore.prm' was used to compute the binding energies. A total of 150 docking poses were generated, ranked according to the scoring function, and the highest scoring pose was used in further analyses.

**Molecular dynamics simulations of ARC-docked mortalin and p53 structure.** MD simulations were carried out using the GROMACS package (33,34). The force field Gromos43a1 (35) was used for ARC-docked mortalin structure (33,36,37). The GROMACS topology file was generated using the antechamber python parser interface (ACPYPE) script. The docked protein structure was solvated in a cubic box. Water molecules and appropriate counter-ions were added to neutralize the system. The solvated system was minimized using steepest descent and conjugate gradient methods until the force on each atom was less than 100 kJ/mol/nm. These geometry minimized systems were used for 10 nsec for carrying out isobaric (constant pressure-temperature) MD simulations. The temperature and pressure of the system was maintained at 300 K and 1 atmosphere pressure, respectively, with a time constant of 5 psec. A 2-fsec time step was used for integrating the equations of motion. Particle Mesh Ewald summation method along with periodic boundary conditions were also applied throughout to calculate the electrostatic potential between partial charges on atoms. Visual Molecular Dynamics (VMD) version 1.9.2 was used to calculate the root mean square deviations (RMSD) and hydrogen bond dynamics.

**Cell culture.** HT1080 (fibrosarcoma), A549 (lung carcinoma) and U2OS (osteosarcoma) human cell lines were purchased from DS Pharma Biomedical Co., Ltd., Osaka, Japan, and cultured in complete Dulbecco's modified Eagle's medium (DMEM; Life Technologies) medium using normal cell culture conditions with 10% fetal bovine serum as a supplement at 37°C, 5% CO<sub>2</sub> and 95% air in a humidified incubator. The cultured cells were maintained for 6-10 passages. ARC (Wako Pure Chemical Industries, Ltd., Osaka, Japan), GPSE and GPSE:50% $\gamma$ CD complex were dissolved in dimethylsulfoxide (DMSO), and directly added to the cell culture medium to obtain the working concentrations (as indicated in the respective figures).

**Preparation of the GPSE-50% $\gamma$ CD complex.** The complex of green propolis supercritical extract (GPSE) with  $\gamma$ CD was prepared by a conventional kneading method. GPSE and  $\gamma$ CD were mixed in a small amount of water. The slurry was kneaded until it became a homogeneous paste. After freeze-drying of the paste, the GPSE- $\gamma$ CD complex thus obtained was used in the present study. The levels of artepillin in GPSE, and its complex with  $\gamma$ CD, were determined by high-performance liquid chromatography (HPLC) using the Shimadzu HPLC system (LC-2010C; Shimadzu Corp., Kyoto, Japan) as previously described (38). A Phenomenex HPLC column [Luna 5u C18(2) 100A: 4.60 mm I.D. x150 mm] was used and the fractionation was performed at 40°C using solution A: H<sub>2</sub>O (0.5% acetic acid) and solution B: Acetonitrile with gradient program as follows: Isocratic 70% A (0-5 min), linear gradient 70%  $\rightarrow$  0% A (5-30 min); flow rate, 1 ml/min; injection volume, 10  $\mu$ l. Detection was performed at 320 nm (Shimadzu HPLC system LC-2010C; Shimadzu Corp.).

**Cell proliferation assay.** Cytotoxicity assay was performed using 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay (Life Technologies/Thermo Fisher Scientific, Waltham, MA, USA) in which cell viability was observed by the conversion of yellow MTT by the mitochondrial dehydrogenases of living cells into purple formazan (39). The statistical significance of the results was determined from 3-4 independent experiments including triplet or quadruplet sets in each experiment.  $IC_{50}$  values were calculated by the linear regression methods in MS excel.

**Morphological observation.** The cells were seeded in 12-well plates and treated with various concentrations of ARC (100-400  $\mu$ M, as indicated in Fig. 2C), GPSE and GPSE-50% $\gamma$ CD (0.5%). Morphological changes were observed under a phase contrast microscope (Nikon Eclipse TE300; Nikon, Tokyo, Japan) after 48 h.

**Colony formation assay.** The cells (500 cells/well) were seeded in a 6-well plate. Following 24 h of incubation, when the cells had attached to the surface of the dish they were treated with ARC, GPSE or GPSE-50% $\gamma$ CD (0.1%) in culture medium (DMEM). The cultures were maintained for 10-15 days (when colonies formed in the control cultures) with a regular change of medium every 3rd day. The colonies were fixed in acetone: methanol (1:1), and stained with crystal violet (Wako Pure Chemical Industries Ltd.) at the end of experiments.

**Immunofluorescence staining.** The cells were seeded on glass coverslips placed in 12-well plates and treated with either ARC (300  $\mu$ M), GPSE or GPSE-50% $\gamma$ CD (0.5%) in DMEM. After 48 h of treatment, cells were fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS), permeabilized with 0.1% Triton X-100 for 10 min, blocked with 0.2% bovine serum albumin (BSA)/PBS for 1 h and incubated with specific primary antibodies including anti-mortalin (clone 088: raised in our laboratory) (3-5  $\mu$ g/ml) and p53 (DO-1: SC-126) (3-5  $\mu$ g/ml) (Santa Cruz Biotechnology, Santa Cruz, CA, USA) at 4°C overnight. The cells were then incubated with either Alexa-488 or Alexa-594 conjugated secondary antibodies (1  $\mu$ g/ml) (A11034 or A11032, Molecular Probes, Eugene, OR, USA) for 1 h. Counterstaining was performed with Hoechst 33342 (Sigma, St. Louis, MO, USA) in the dark for 10 min. The cells were examined on a Zeiss Axiovert 200 M microscope and analyzed using AxioVision 4.6 software (Carl Zeiss, Oberkochen, Germany).

**Wound healing assay.** The cells were plated in 6-well plates and allowed to make monolayers following which a scratch was made using a 100- $\mu$ l pipette tip. The cells were then washed with PBS and cultured with the control or test compounds [GPSE or GPSE-50% $\gamma$ CD or  $\gamma$ CD, (0.1%) as indicated in Fig. 4C]. The movement of the cells to the scratch area was followed for the following 24-48 h. Images were acquired under a phase contrast microscope (Nikon) with a X10 phase objective lens at the 0, 24 and 48 h time-points. The movement was quantitated by measuring the empty surface area in the control and test samples by ImageJ software. The results are expressed as the means  $\pm$  standard deviation of the mean (SEM). Statistical significance of the data was deter-

mined using a Student's t-test. Values of  $P < 0.05$ ,  $P < 0.01$  and  $P < 0.001$  were considered to indicate significant, very significant and highly significant differences, respectively.

**In vivo tumor progression.** The effects of GPSE and GPSE-50% $\gamma$ CD on *in vivo* tumor progression were investigated in a nude mouse subcutaneous tumor xenograft model. BALB/c nude mice (4 weeks old, female, 3 mice in each group/average weight 21.8 g) were obtained from Nihon Clea (Tokyo, Japan). The animals were allowed to acclimatize in our laboratory for 1 week. The cells were injected subcutaneously ( $1 \times 10^7$  suspended in 0.2 ml of growth medium) into the abdomen of the nude mice and the tail vein ( $1 \times 10^6$  suspended in 0.2 ml of growth medium). GPSE and GPSE 50% $\gamma$ CD were administered (by oral gavage) every alternate day beginning at 1 day after the injection of the cells for 3 weeks. Tumor formation and the body weight of the mice were monitored every alternate day. The volume of the subcutaneous tumors was calculated as  $V = L \times W^2/2$ , where 'L' was the length and 'W' was the width of the tumor, respectively. Statistical significance of the data was calculated from 3 independent experiments ( $n=3$  per experiment). All procedures were carried out in accordance with the Animal Experiment and Ethics Committee, Safety and Environment Management Division, National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba, Japan.

**Statistical analysis.** All the experiments were performed in triplicate and data are expressed as the means  $\pm$  SEM. An unpaired t-test (GraphPad Prism GraphPad Software, San Diego, CA, USA) and one-way ANOVA followed by Tukey's honestly significant difference (HSD) post hoc test were used to determine the degree of significance between the control and experimental samples. Statistical significance was defined as P-values as follows:  $P < 0.05$  (significant),  $P < 0.01$  (very significant) and  $P < 0.001$  (highly significant) for the t-test and  $P < 0.05$  for ANOVA followed by a post hoc test, as indicated in the figure legends.

## Results

**ARC docks into the mortalin-p53 complexes and induces the activation of p53.** The interaction of mortalin and p53 occurs at N-terminal amino acids residues (253-282) of mortalin and C-terminal residues (323-337) of p53 (12,40-42). Based on this information, we docked ARC with mortalin or p53 or at mortalin-p53 complex interface. ARC interacted with the crystal structure of mortalin and p53 at the binding regions. It showed a docking score of -6.99 kcal/mol with mortalin. The docked complex exhibited multiple hydrogen bonds and hydrophobic interactions (Fig. 1A-a). The ligand formed 3 hydrogen bonds, 2 with Arg 126 and 1 with Thr 271 of mortalin, while residues Glu 132, Ala 195, Tyr 196, Thr 267, Asn 268 and Gly 269 played a role in hydrophobic interactions. The second oxygen atom of ARC formed 2 hydrogen bonds with nitrogen atoms (NH1 and NH2) of Arg 126. The third hydrogen bond was formed between the third oxygen atom of ARC and the third oxygen atom of Thr 271. Together the hydrogen bonds and hydrophobic interactions formed a stable protein-ligand complex.

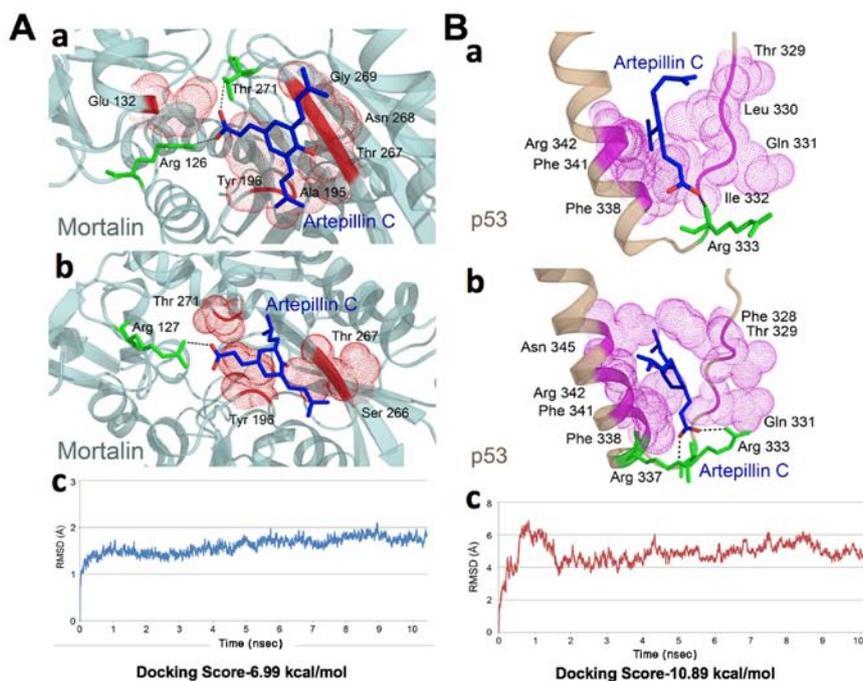


Figure 1. Artepillin C (ARC) docks into the mortalin-p53 complex. (A) Molecular docking of ARC with mortalin-p53 crystal structure (a). ARC is shown in blue, mortalin in grey and p53 in pink. Amino acids involved in the interaction are labeled in each panel. The stability of the ARC-docked mortalin structure and their RMSD trajectory (b and c) is shown. (B) Molecular docking of ARC with p53 (a). The stability of the ARC-docked p53 structure and their RMSD trajectory (b and c) is shown.

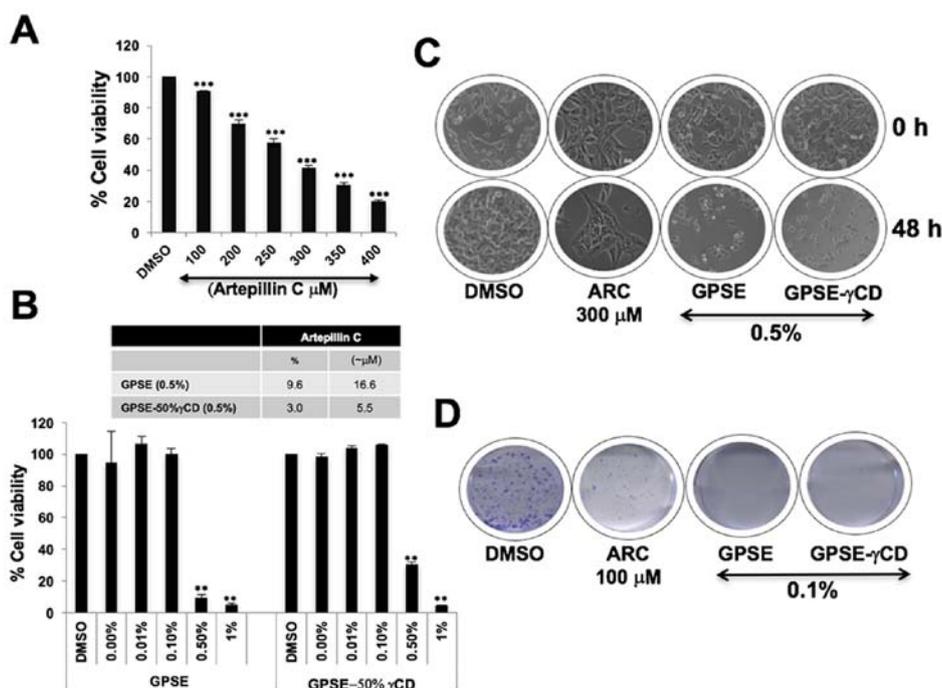


Figure 2. Cytotoxicity of artepillin C (ARC) to human cancer cells. Cell viability assay showing the (A) ARC-, and (B) GPSE and GPSE-50% $\gamma$ CD-induced decrease in HT1080 cell viability. (C) Cell morphology images suggesting growth arrest (in ARC-treated) and apoptosis (in GPSE- and GPSE- $\gamma$ CD treated) cells are shown. (D) The inhibition of colony formation in HT1080 cells in response to long-term treatment with ARC, GPSE and GPSE- $\gamma$ CD. GPSE and GPSE- $\gamma$ CD (0.1%) exerted a more potent effect than ARC (100  $\mu$ M). \*\*\* $P$ <0.001 (highly significant) and \*\* $P$ <0.01 (very significant) are shown as compared to the control.

The docking of ARC with p53 exhibited a score of -10.89 kcal/mol. The ligand was observed to form 1 hydrogen bond and various hydrophobic interactions (Fig. 1B-a). The nitrogen atom of Arg 333 was observed to form a hydrogen

bond with the third oxygen atom of ARC. Residues Thr 329, Leu 330, Gln 331, Ile 332, Phe 338, Phe 341 and Arg 342 were involved in the hydrophobic interaction with p53. ARC docked in the cavity of p53 forming a stable complex.

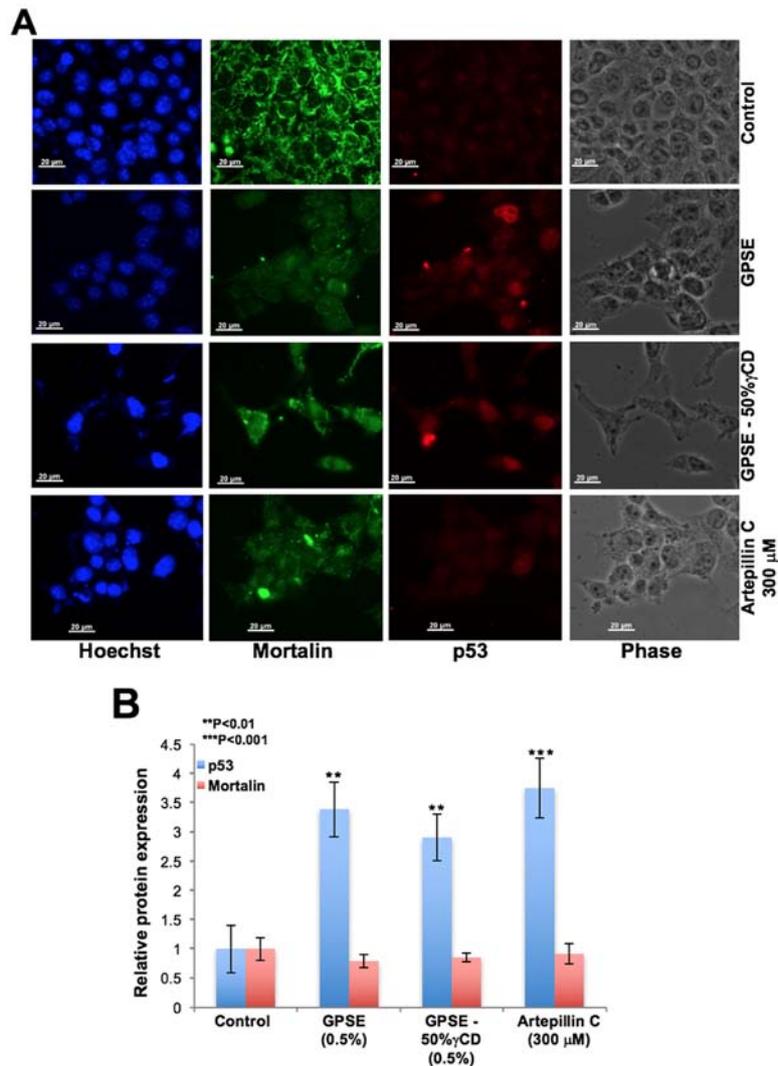


Figure 3. Artepillin C, GPSE and GPSE- $\gamma$ CD induce the nuclear translocation and activation of p53. (A) Immunostaining showing an increase in nuclear p53 in the cells treated with ARC, GPSE and GPSE- $\gamma$ CD, as compared to the control. (B) Quantification from 3 independent experiments and statistical significance is shown. Statistical significance of the data \*\*\*P<0.001 (highly significant) and \*\*P<0.01 (very significant) are shown as compared to the control.

*Molecular dynamics simulations and energy stabilization of ARC in the mortalin-p53 complex.* The stability of the ARC-docked mortalin structure was further analyzed by simulating the complex for 10 nsec under conditions mimicking the bodily environment. The complex was observed to be stable for a period of 5 nsec from 5 to 10 nsec, as seen in the RMSD trajectory. Changes observed in the interacting residues of mortalin are shown in Fig. 1A-b. One hydrogen bond was observed between the oxygen atom of Arg 127 and the third oxygen atom of ARC, while residues Tyr 196, Ser 266, Thr 267 and Thr 271 were involved in hydrophobic interaction following simulations. Despite the changes in the interaction pattern of ligand-docked protein complex, the structure was more stable following simulations. The RMSD trajectory is shown Fig. 1A-c. A change in the interaction pattern of ARC docked p53 complex was observed (Fig. 1B-b). The number of hydrogen bonds between ARC and p53 increased to 2 post-simulations. One bond was formed between the second oxygen atom of ARC and nitrogen atom (NH1) of Arg 333, while the second was observed to be formed between nitrogen atom (NE) of Arg 337. The number of residues involved in hydrophobic

interactions were the same, with 2 residues (Leu 330 and Ile 332) replaced with Phe 328 and Asn 345. The complex was more stable post-simulations. The ARC-docked p53 structure was observed to be stable from 5 to 10 nsec that can be seen in the RMSD trajectory (Fig. 1B-c). Bioinformatics analyses revealed that the ARC docked into the mortalin-p53 complexes (Fig. 1), similar to CAPE, and hence may cause abrogation of these complexes, and the re-activation of p53 function in cancer cells.

*Anticancer activity of ARC, GPSE and the GPSE- $\gamma$ CD complex.* We then examined the cytotoxicity of ARC in HT1080 cells and found that it induced a considerable (>20%) decrease in cell viability at concentrations of 250  $\mu$ M (IC<sub>50</sub>, 275  $\mu$ M) (Fig. 2A). Based on such high IC<sub>50</sub> values, we considered whether the extract of green propolis could be more effective than purified ARC for the reasons including that: i) it may provide a complex mixture of bioactives with multi-module action; and ii) it may protect the bioactive components from degradation. We prepared the GPSE and its conjugate with  $\gamma$ CD (GPSE- $\gamma$ CD) and HPLC analyses revealed that they

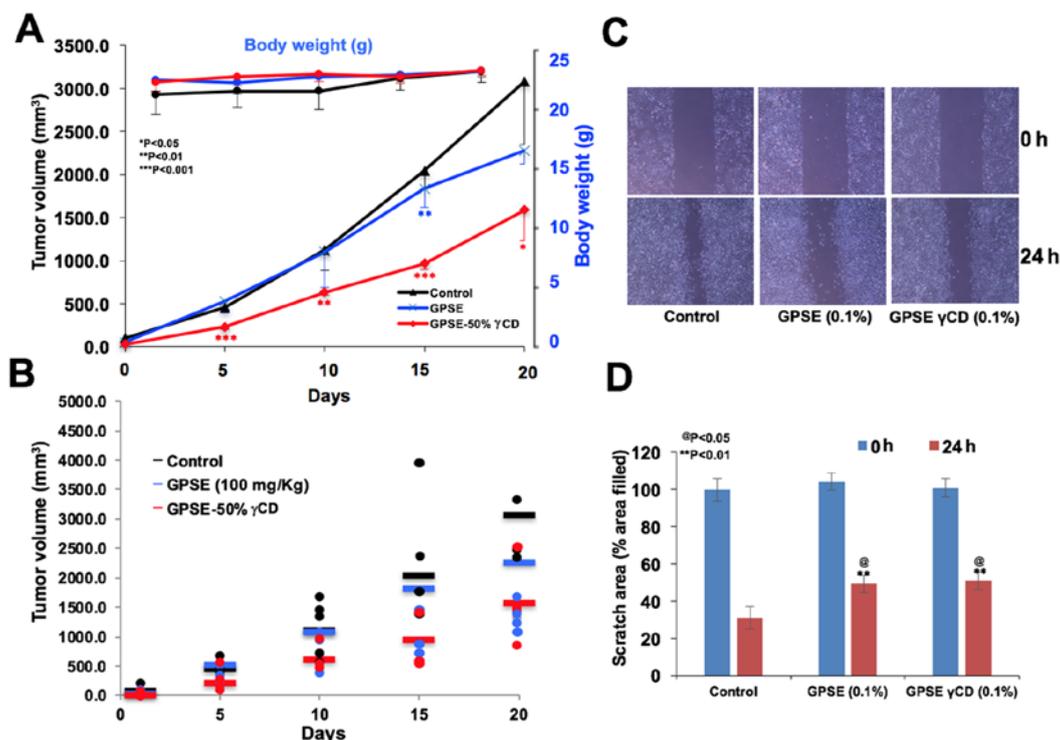


Figure 4. GPSE and GPSE- $\gamma$ CD possess antitumor and anti-migratory activities. (A) Nude mice treated with GPSE and GPSE- $\gamma$ CD showed no toxicity, as their body weight remained unaltered. (B) Inhibition of tumor growth in subcutaneous tumor xenografts was observed in mice fed GPSE and GPSE- $\gamma$ CD. The mice fed GPSE- $\gamma$ CD exhibited a greater suppression of tumor growth both in the (A) average and (B) individual mouse tumor volume analyses;  $n > 6$  from each group. (C) Wound-scratch assay revealed the anti-migratory activity of the GPSE extract which led to a slower migration rate of the treated cells into the scratch area as compared to the control. (D) Quantification of the migratory ability of the cells cultured in the control and GPSE-supplemented medium is represented as the covered area calculated from 3 experiments. Three-four independent random images were captured. The area at 0 h was taken as the control (set at 100%). Statistical significance is indicated as follows: \* $P < 0.01$ , very significant as determined by the t-test and <sup>@</sup> $p < 0.05$ , significant as determined by ANOVA followed by Tukey's honestly significant difference (HSD) post hoc test.

contained 9.6 and 3.0% ARC, respectively. The  $IC_{50}$  values were 0.2–0.5% for GPSE and GPSE- $\gamma$ CD, whereas they were 250–300  $\mu$ M for ARC (Fig. 2B). Similar results were obtained with the A549 and U2OS cells (data not shown). Cell morphological analysis revealed that the cytotoxicity induced by 0.5% GPSE and GPSE- $\gamma$ CD was comparable to that induced by 300  $\mu$ M ARC (Fig. 2C). Long-term colony forming assays revealed that ARC (100  $\mu$ M) induced a marked decrease in the number and size of the colonies; a more potent decrease was observed by treatment with 0.1% GPSE and GPSE- $\gamma$ CD (Fig. 2D).

We then examined the above-mentioned concentrations of ARC, GPSE and GPSE- $\gamma$ CD, and determined their effects on mortalin-p53 interactions as predicted by bioinformatics analysis (Fig. 1). In several independent experiments, we found that treatment with GPSE and ARC led to the nuclear translocation of p53 (Fig. 3A), accompanied by an increase in p53 expression (Fig. 3B). We performed antitumor assays using a subcutaneous tumor xenograft model with nude mice. As shown in Fig. 4, mice fed the GPSE or GPSE- $\gamma$ CD exhibited no change in body weight (Fig. 4A) or physical activity (data not shown) with respect to the control group. A reduction in tumour growth was observed in the mice fed GPSE or GPSE- $\gamma$ CD as compared to the control group over the 3 weeks of treatment (Fig. 4B). The tumor growth data were strongly suggestive that the GPSE- $\gamma$ CD induced a more marked suppressive effect on tumor growth, endorsing its potential as an antitumor natural

compound. We also performed *in vitro* wound healing assays to examine whether the GPSE and GPSE- $\gamma$ CD extracts possess anti-migratory activity and to determine whether they can be used to inhibit cancer metastasis. As shown in Fig. 4C and D, a considerable inhibition of cell migration was detected in the cells cultured in the presence of non-toxic doses of GPSE and GPSE- $\gamma$ CD.

## Discussion

A number of different methods have been used to extract active components from propolis, including solvent extraction, e.g., ethanol (3), water or diluted aqueous ethanol (43) and supercritical carbon dioxide extracts (8,44,45). Apart from differences in the yield due to the function of temperature, pressure and solvent concentration, the different extraction methods generate mixtures with different components and also show different activities. Supercritical extracts have non-polar characteristics that are tolerant to oxidation and provide high yields (8,44,46). However, due to their poor solubility in aqueous solvents, their use is limited in *in vitro* and *in vivo* studies. Cyclodextrins (CDs), with inner lipophilic cavities and hydrophilic outer surfaces, are capable of encapsulating molecules by non-covalent inclusion (47,48). We previously used  $\gamma$ CD to conjugate with CAPE to generate a CAPE- $\gamma$ CD complex that showed significant anticancer activity (12). In the present study, we demonstrate that ARC, an active component

of Brazilian green propolis, activates p53 tumor suppressor protein by abrogating its complex with mortalin (p53 inactivating protein), and thus possesses anticancer activity. However, it turned out to be a low efficacy compound (IC<sub>50</sub>, 275 μM). We hypothesized that crude extracts of green propolis may possess better efficacy. Hence, we generated its supercritical extract (GPSE) and its complex with γCD (GPSE-γCD). We herein report that the GPSE containing 9.6% ARC possesses high cytotoxicity to cancer cells, and was effective at concentrations of 0.25 to 0.5% (8.3-16.6 μM ARC). Furthermore, the GPSE-γCD complex (containing 3% ARC) exhibited greater cytotoxicity *in vitro* (0.25 to 0.5% = 2.7-5.5 μM ARC) and greater antitumor activity *in vivo*. Anti-migration assays revealed that GPSE, as well as the GPSE-50%γCD complex, at non-toxic concentrations, caused the delayed migration of cells to the scratch in wound healing assays, suggesting its potential for the treatment of metastatic cancers. Based on these data, GPSE-γCD is proposed as a Natural Efficient and Welfare (NEW) antitumor composite. Further studies are warranted to elucidate the molecular mechanism(s) of the potent anticancer activity of GPSE and its complex with γCD.

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### Competing interests

The authors declare that they have no competing interests.

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