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# Polycarpol from roots of *Melodorum fruticosum* enhances antiproliferative activities of chemotherapeutic drugs against human cervical cancer cells

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**ABSTRACT**: Cervical cancer is the most reported cases of the global public health concern and a leading cause of female death worldwide. Chemotherapy plays a key role in tumor control, but a single drug treatment often results in drug resistance and side effects. This study aimed to investigate the combination effects of polycarpol, the phytochemical compound isolated from roots of *Melodorum fruticosum*, and current chemotherapeutic drugs (5-fluorouracil (5-FU) and cisplatin) against human cervical cancer HeLa cells. The antiproliferative activities of single and combined drugs against HeLa cells were assessed by the MTT assay. The drug interaction effects were studied using the Chou-Talalay method in which the combination index (CI) and dose reduction index (DRI) values were determined. Cell viabilities (%) were determined to assess antiproliferative effects in single and combined drug treatments at 24, 48, and 72 h. The dose- and time-dependent antiproliferative effects of polycarpol were observed. Polycarpol demonstrated a remarkable cytotoxic effect ($IC_{50}$ of $5.27 \pm 2.39$ µM) towards HeLa cells at 48 h, compared with 5-FU ($16.49 \pm 3.04$ µM) and cisplatin ($12.51 \pm 1.16$ µM). The drug interactions were evaluated at sub-toxic doses ($IC_{20}$, $IC_{30}$, and $IC_{40}$) of 5-FU and cisplatin. The polycarpol drug combinations indicated synergistic effect with 5-FU ($IC_{40}$) at 48 h exposure and additive effects with cisplatin ($IC_{20}$ and $IC_{30}$) at 24 h exposure. These findings highlight a potential of polycarpol from roots of *M. fruticosum* as an anticancer agent for treatments of cervical cancer both in single and combined drug treatments.

**KEYWORDS**: cervical cancer, polycarpol, *Melodorum fruticosum*, anticancer, drug interaction

## INTRODUCTION

Cancer is a prevalent genetic disease and a significant global public health challenge, being the important problem cause of death in numerous countries. In Thailand, cervical cancer is the second most common cancer after breast cancer of Thai women [1]. The leading cause of cervical cancer is the infection with the human papillomavirus (HPV) which can be transmitted through sexual contact. Nowadays, utilizing HPV vaccines can effectively reduce the incidence of viral epidemics and associated risk factors of cervical cancer. However, it is still a major problem in many low- and middle-income countries in Asia and Africa [2]. The prevention of HPV through vaccine appears to lack access, affordability, money, awareness, education, quality infrastructure, logistics, and healthcare systems [3].

Cancer chemotherapy plays a major role in a standard treatment to reduce tumoral cell growth or inhibit the cell cycle. Chemotherapy affects both tumoral and normal cell growth and induces the development of drug resistance and several side effects [4]. Therefore, development of new drugs in the concern of an inexpensive cost and less toxicity for cancer therapy is in urgent need. Natural products derived from natural sources are regarded as a potential source of pharmaceutical therapeutics, particularly with their potential for anticancer activities [5]. Thus, natural products are an alternative to increase the efficiency of cancer treatment. Numerous natural products such as peanut testa extracts [6], taxol [7], and alkaloid compounds (berberine and palmatine) [8] have demonstrated potential for cancer therapy. Nowadays, natural products become an intriguing focus on enhancing anticancer activities of current anticancer drugs or lessening problems of side effects and drug resistance from chemotherapy through drug combination treatments [9, 10].

5-Fluorouracil (5-FU), a pyrimidine analog, is one of the current anticancer drugs that is widely used in various cancer treatments. 5-FU mechanism of action is based on interfering the DNA and RNA synthesis via folate metabolic pathway through inhibiting the action of thymidylate synthase and inducing the cytotoxic metabolite to misincorporate into RNA and DNA [11]. Despite several advantages of 5-FU, its clinical application including chemotherapy for cervical cancer was limited because the drug resistance was developed after chemotherapy. The side effects in cancer patients are the same as a general chemotherapy drug, especially cardiotoxicity [12]. Similarly, the current anticancer drug cisplatin (*cis*-diamminedichloroplatinum (II)) is preferably used in cervical cancer therapy. Cisplatin mechanism of action is through its binding to nuclear DNA to form DNA adducts includ-

ing mono-adduct, inter-strand, and intra-strand cross-links, which subsequently interfere with normal transcription, and/or DNA replication mechanisms. Consequently, cisplatin could induce DNA damage, inhibit DNA repair mechanism, and promote apoptosis in cancer cells [13]. However, cisplatin used in cervical cancer treatment has been shown to cause drug resistance and several undesirable side effects [13, 14]. Accordingly, new effective drugs that may reduce the side effects of chemotherapy are urgently needed.

*Melodorum fruticosum*, white cheesewood or devil tree (commonly known in Thai as Lamduan), is an herbal plant found in Southeast Asia [15]. Phytochemical compounds found in many plant parts of *M. fruticosum* include numerous aromatic compounds, flavonoids, heptenoids, alkaloids, terpenoids, and polycarpol [16]. Polycarpol is a lanostane triterpene found in Annonaceae family plants that possesses several pharmaceutical activities such as antimicrobial, antifilarial, antiinflammatory, and antitumor activities [17]. Recently, our screening studies for anticancer agents revealed that polycarpol (100 µg/ml) isolated from roots of *M. fruticosum* showed antiproliferative activity (viability = 34%) against cervical cancer HeLa cells by MTT assay and exhibited a less toxicity against the noncancer Vero cells (viability = 66%), indicating a selectivity against cervical cancer cells. Therefore, in this study, polycarpol is evaluated further for its antiproliferative activity against cervical cancer HeLa cells in comparison with 5-FU and cisplatin. In addition, its antiproliferative activity in combinations with 5-FU and cisplatin is also investigated.

## MATERIALS AND METHODS

### Materials

The HeLa cells was obtained from the National Cancer Institute, Bangkok, Thailand. RPMI-1640 medium (Lot no. 2725192) was purchased from Gibco-BRL Life-Sciences (Basel, Switzerland). Fetal bovine serum (FBS) (Lot no. 2575610), penicillin G, and streptomycin (Lot no. 15140-122) were purchased from Gibco-BRL (New York, NY, USA). The 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyl tetrazolium bromide (MTT) (Lot no. 736779) was purchased from Invitrogen (Carlsbad, CA, USA). Dimethyl sulfoxide (DMSO) (Lot no. 35010523) was purchased from PanReac AppliChem (Castellar del Valles, Barcelona, Spain). Trypsin-EDTA (Lot no. 2455123) was purchased from Gibco-BRL. 5-FU (Lot no. 1J009942) was purchased from PanReac Applichem. Cisplatin (Lot no. 2210000) was purchased from EDQM (Strasbourg, France).

### Polycarpol extraction and purification

Polycarpol was extracted and purified from roots of *M. fruticosum* according to the method described by Jung et al [18]. In brief, the root powder of *M. fruticosum* (3.0 kg) was subjected to extraction by using ethyl acetate (EtOAc) (3 × 10 l) at room temperature. The crude EtOAc extract (yield of 45.0 g, 1.5%) was fractionated over silica gel column chromatography (CC) and eluted with a gradient system of hexane:EtOAc and EtOAc:MeOH. Fractions with similar TLC patterns were combined to yield 10 pooled fractions, MF1–MF10. Fraction MF10 was separated over silica gel CC and eluted with a gradient system of hexane:EtOAc to give 6 subfractions (MF10.1–MF10.6). Subfraction MF10.2 was further purified by silica gel CC and eluted with an isocratic system of hexane:EtOAc (80:20) to afford polycarpol as colorless needles. The purified polycarpol was stored in the amber glass vial and kept at 4 °C for future use. Chemical structure and HPLC profile of polycarpol are presented in supplementary Fig. S1 and Fig. S2, respectively. The purity of a purified polycarpol used throughout this study was 94.29% (Fig. S2).

### Cell culture condition

The HeLa and Vero cells were cultured in RPMI-1640 medium supplemented with 10% FBS, 100 U/ml penicillin, and 100 µg/ml streptomycin (Gibco). The cells were incubated at 37 °Cin a humidified atmosphere containing 5% $CO_2$. The exponentially growing cells were used for all subsequent experiments.

### *In vitro* antiproliferation activity

The percentage of cell viability after treatment with polycarpol, 5-FU, or cisplatin was determined by MTT assay [19]. The cell suspension (100 µl) was seeded into 96-well plates at a density of $8 \times 10^3$ cells/well and incubated at 37 °C in a humidified atmosphere containing 5% $CO_2$ for 24 h. Subsequently, cells were treated with various concentrations of polycarpol, 5-FU, or cisplatin and incubated for 24, 48, and 72 h. The solvent mixture (DMSO:Absolute ethanol, 0.5%:0.5%, v/v) was used as a solvent control.

At a specified exposure time, the medium was removed and then the 100 µl of fresh medium containing 1.2 mM MTT (10 µl/well) was added to each well. After incubation for 2.5 h, the formazan crystal product was dissolved with 100 µl DMSO in a dark room at room temperature for 15 min. The absorbance (A) of formazan was measured using microplate reader (Bio-Rad Laboratories, Hercules, CA, USA) at the wavelength of 570 nm, and a reference wavelength of 655 nm was used to subtract optical density (OD) of cellular debris at 570 nm. The following equation was used to calculate cell viability:

$$\% \text{ Cell viability} = (A_{570} \text{ sample} - OD_{655} \text{ sample}) / (A_{570} \text{ control} - OD_{655} \text{ control}) \times 100,$$

where A and OD are the absorbance and optical density, respectively.

### Selectivity index (SI)

The degree of selectivity of the compounds was expressed by its SI value, in which the SI value $> 2$ is considered selective toxicity against cancer cells, while the SI value $< 2$ is considered general toxicity against both cancer cells and normal cells [20]. The SI value was calculated using the equation:

$$\text{SI} = \text{IC}_{50} \text{ noncancer cell}/\text{IC}_{50} \text{ cancer cell}.$$

### Determination of drug interaction

The combination index (CI) was calculated according to the median-effect principle to estimate the interactions between polycarpol with current chemotherapy drugs (cisplatin and 5-FU) [21]. Drug combination treatments were performed at exposure times of 24, 48, and 72 h by varying doses of polycarpol and fixing the dose of the current anticancer drug (cisplatin or 5-FU) at a sub-toxic dose ($\text{IC}_{20}$, $\text{IC}_{30}$, or $\text{IC}_{40}$). $\text{IC}_{50}$ values of polycarpol from the drug combination treatments were determined, and CI values were calculated following the equation below:

$$\text{CI} = \text{D1}/\text{Dx1} + \text{D2}/\text{Dx2} + \alpha(\text{D1}\,\text{D2})/(\text{Dx1}\,\text{Dx2}),$$

where D1 is a dose of drug 1 (5-FU or cisplatin) to produce 50% growth inhibition in D2 combination, D2 is a dose of drug 2 (polycarpol) to produce 50% growth inhibition in D1 combination, Dx1 is a dose of drug 1 (5-FU or cisplatin) to produce 50% growth inhibition alone, Dx2 is a dose of drug 2 (polycarpol) to produce 50% growth inhibition alone, and $\alpha = 1$ for mutually nonexclusive modes of drug actions (independent mode of action). The CI values are CI $> 1.3$, antagonism; CI $= 1.1$–$1.3$, moderate antagonism; CI $=$ 0.9–1.1, additive effect; CI $= 0.8$–$0.9$, slight synergism; CI $= 0.6$–$0.8$, moderate synergism; CI $= 0.4$–$0.6$, synergism; and CI $= 0.2$–$0.4$, strong synergism.

The fold of dose reduction in drug combination treatment was calculated and expressed as a DRI value that explains the level of drug combination effect compared to the single drug treatment. The DRI was calculated by using the following equation:

$$\text{DRI} = \text{Dx}/\text{D},$$

where D is a dose of a drug combined with the other drug to produce 50% cell viability, while Dx is a dose of a drug alone to produce 50% cell viability. DRI value more than 1-fold is favored dose reduction whereas the value less than 1-fold is disfavored dose reduction.

### Statistical analysis

All the data are from 3 independent experiments representing the mean ± standard error of the mean (SEM). The data were analyzed using the statistical program IBM SPSS version 28.0 for Windows. One-way ANOVA analysis was tested to compare significant differences with Tukey's post hoc test. The statistically significant differences were considered at $p < 0.05$.

## RESULTS

### Antiproliferative activities of polycarpol, 5-FU, and cisplatin against a cervical cancer cell line

The $\text{IC}_{50}$ values of polycarpol, 5-FU, and cisplatin at 24, 48, and 72 h were evaluated in the single drug treatments. HeLa cells were exposed to different concentrations of polycarpol, 5-FU, and cisplatin, and MTT assay was used to determine the viability of the cells. MTT results showed that polycarpol, 5-FU, and cisplatin significantly inhibited proliferation of HeLa cells in a dose- and time-dependent manner (Fig. 1). Polycarpol inhibited proliferation of HeLa cells ($\text{IC}_{50} = 100.59 \pm 15.72$, $5.27 \pm 2.39$, and $3.52 \pm 1.09$ µM at 24, 48, and 72 h exposures, respectively) (Fig. 1A,B) more effective than that of the noncancer Vero cells ($\text{IC}_{50} = 226.91 \pm 12.13$, $68.80 \pm 8.13$, and $49.59 \pm 8.65$ µM at 24, 48, and 72 h exposures, respectively) (Table 1), indicating that the HeLa cell line was more sensitive to polycarpol than the noncancer Vero cell line. 5-FU inhibited the growth of HeLa cells with $\text{IC}_{50}$ values of $> 200$, $16.49 \pm 3.04$, and $2.91 \pm 0.12$ µM at 24, 48, and 72 h exposures, respectively (Fig. 1C,D), while cisplatin suppressed the growth of HeLa cells with $\text{IC}_{50}$ values of $34.61 \pm 0.86$, $12.51 \pm 1.16$, and $8.27 \pm 2.20$ µM at exposure times of 24, 48, and 72 h, respectively (Fig. 1E). Polycarpol exhibited the most efficient cytotoxicity at 48 h exposure (Fig. 1B), while cisplatin and 5-FU showed the most efficient cytotoxicity at 24 h exposure (Fig. 1E) and 72 h exposure (Fig. 1D), respectively, against a cervical HeLa cell line.

### Selectivity index (SI) of polycarpol, 5-FU, and cisplatin against a cervical cancer cell line

The degree of selectivity of the anticancer drug was expressed by its SI value [20]. The immortalized noncancer Vero cell line was used as a control in the selectivity analysis due to its non-tumorigenic origin. The *in vitro* activities of polycarpol, 5-FU, and cisplatin on the Vero cell line were used to estimate SI as described in the Materials and Methods section. As shown in Table 1, polycarpol presented the best SI at 48 h and 72 h exposures (13.06 and 14.09, respectively) and could be considered a selective compound against a cervical HeLa cell line. In addition, polycarpol also showed a greater SI (2.26) than that of cisplatin (1.11) against HeLa cell line at 24 h exposure. Despite its effective anticancer activity, cisplatin exhibited SI values $< 2$ at all exposure times against HeLa cells, which could be considered as non-selective.

### Antiproliferative activities of polycarpol in combination treatments with current anticancer drugs (cisplatin and 5-FU) against cervical cancer cells

To elucidate the potential of polycarpol for enhancing the antiproliferative activities of current chemotherapeutic drugs (cisplatin and 5-FU) against HeLa cells, we performed combination treatments of polycarpol

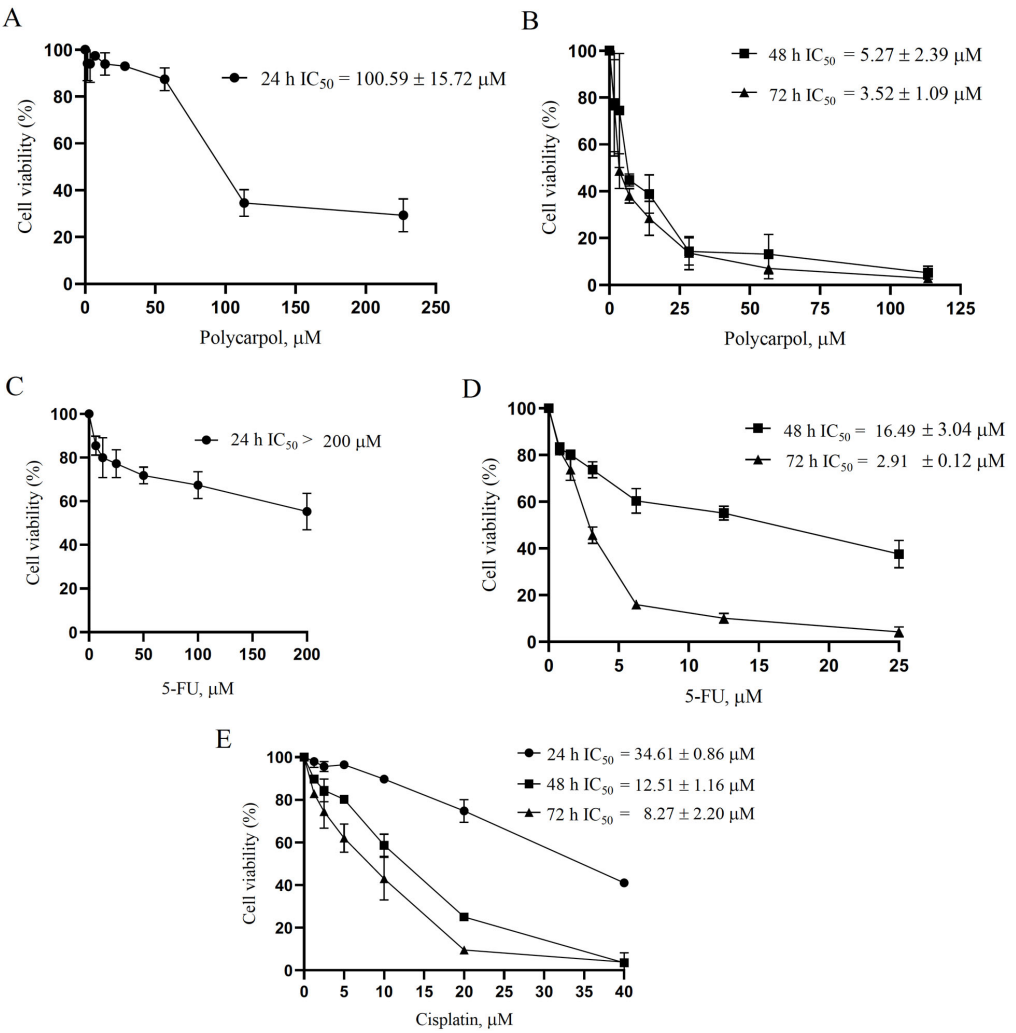


**Fig. 1** Antiproliferative effects of single agent treatments on the cervical cancer HeLa cells. A and B, polycarpol; C and D, 5-FU; and E, cisplatin. Cells were treated with various concentrations of polycarpol, 5-FU, and cisplatin for 24, 48, and 72 h. The percentages of cell viability were calculated relative to the solvent control (0.50% ethanol + 0.50% DMSO) treatment. Data are presented as mean ± SEM for 3 independent experiments.

**Table 1** Selectivity Index (SI) of polycarpol, 5-FU, and cisplatin.

| Anticancer agent | Exposure Time | $IC_{50}$ HeLa cell, µM (mean ± SEM) | $IC_{50}$ Vero cell, µM (mean ± SEM) | SI |
|---|---|---|---|---|
| Polycarpol | 24 h | 100.59 ± 15.72 | 226.91 ± 12.13 | 2.26 |
| | 48 h | 5.27 ± 2.39 | 68.80 ± 8.13 | 13.06 |
| | 72 h | 3.52 ± 1.09 | 49.58 ± 8.65 | 14.09 |
| 5-FU | 24 h | >200 | >1000 | ND |
| | 48 h | 16.49 ± 3.04 | 167.70 ± 17.38 | 10.17 |
| | 72 h | 2.91 ± 0.12 | 8.61 ± 0.52 | 2.96 |
| Cisplatin | 24 h | 34.61 ± 0.86 | 38.33 ± 1.35 | 1.11 |
| | 48 h | 12.51 ± 1.16 | 13.37 ± 0.09 | 1.07 |
| | 72 h | 8.27 ± 2.20 | 7.40 ± 0.18 | 0.89 |

Data are the means of 3 independent experiments. Selectivity Index (SI) = $IC_{50}$ Vero cell/$IC_{50}$ HeLa cell. SI value > 2 indicates selective toxicity against cancer cells [19]. ND = Not determined.

**Table 2** CI and DRI of the combination treatments between polycarpol, cisplatin, and 5-FU against HeLa cells.

| Exposure time | Parameter used for drug interaction assessment | | | | CI | DRI | | |
|---|---|---|---|---|---|---|---|---|
| | $IC_{50}$ of Polycarpol (μM) | | Sub-toxic dose of Cis (μM) | Sub-toxic dose of 5-FU (μM) | | Cis | 5-FU | Poly-carpol |
| | Alone | Combination | | | | | | |
| | | | $IC_{20}$ | $IC_{20}$ | | | | |
| 24 h | 100.59 ± 15.72 | 17.68 ± 0.35 | 16.93 ± 2.55 | – | 1.08 ± 0.01 | 2.04 | – | 5.69 |
| 48 h | 5.27 ± 2.39 | 4.21 ± 0.02 | 4.38 ± 0.41 | – | 2.80 ± 0.01 | 2.86 | – | 1.25 |
| 72 h | 3.52 ± 1.09 | 3.11 ± 0.10 | 1.98 ± 0.74 | – | 2.71 ± 0.07 | 4.18 | – | 1.13 |
| 24 h | 100.59 ± 15.72 | ND | – | ND | ND | – | ND | ND |
| 48 h | 5.27 ± 2.39 | 9.27 ± 0.57 | – | 1.38 ± 1.27 | 1.99 ± 0.12 | – | 11.95 | 0.57 |
| 72 h | 3.52 ± 1.09 | 6.04 ± 0.57 | – | 0.89 ± 0.54 | 2.55 ± 0.12 | – | 3.27 | 0.58 |
| | | | $IC_{30}$ | $IC_{30}$ | | | | |
| 24 h | 100.59 ± 15.72 | 10.59 ± 1.52 | 22.63 ± 2.74 | – | 1.06 ± 0.12 | 1.53 | – | 9.49 |
| 48 h | 5.27 ± 2.39 | 6.64 ± 0.59 | 7.45 ± 0.47 | – | 5.16 ± 0.41 | 1.68 | – | 0.79 |
| 72 h | 3.52 ± 1.09 | 5.73 ± 1.20 | 3.39 ± 1.40 | – | 5.18 ± 1.67 | 2.44 | – | 0.61 |
| 24 h | 100.59 ± 15.72 | ND | – | ND | ND | – | ND | ND |
| 48 h | 5.27 ± 2.39 | 6.41 ± 0.35 | – | 3.67 ± 0.53 | 1.71 ± 0.08 | – | 4.49 | 0.82 |
| 72 h | 3.52 ± 1.09 | 9.73 ± 2.12 | – | 1.69 ± 0.19 | 4.95 ± 0.95 | – | 1.72 | 0.36 |
| | | | $IC_{40}$ | $IC_{40}$ | | | | |
| 24 h | 100.59 ± 15.72 | 20.74 ± 0.64 | 28.62 ± 1.80 | – | 1.21 ± 0.01 | 1.21 | – | 4.85 |
| 48 h | 5.27 ± 2.39 | 14.45 ± 3.08 | 9.85 ± 1.12 | – | 5.69 ± 1.04 | 1.27 | – | 0.36 |
| 72 h | 3.52 ± 1.09 | 8.83 ± 0.35 | 5.77 ± 1.77 | – | 4.96 ± 0.17 | 1.43 | – | 0.31 |
| 24 h | 100.59 ± 15.72 | ND | – | ND | ND | – | ND | ND |
| 48 h | 5.27 ± 2.39 | 1.37 ± 0.08 | – | 7.70 ± 1.96 | 0.86 ± 0.02 | – | 3.80 | 2.14 |
| 72 h | 3.52 ± 1.09 | 0.99 ± 0.02 | – | 2.32 ± 0.04 | 1.31 ± 0.01 | – | 1.25 | 3.52 |

Polycarpol: a lanostane triterpene isolated and purified from roots of *M. fruticosum*; Cis: cisplatin; and 5-FU: 5-fluorouracil. Antagonism: CI > 1.3; Moderate antagonism: CI = 1.1–1.3; Additive effect: CI = 0.9–1.1; Slight synergism: CI = 0.8–0.9; Moderate synergism: CI = 0.6–0.8; Synergism: CI = 0.4–0.6; and Strong synergism: CI = 0.2–0.4. ND = Not determined.

with sub-toxic doses ($IC_{20}$, $IC_{30}$, $IC_{40}$) of cisplatin or 5-FU. The $IC_{50}$ values of polycarpol in combinations with a sub-toxic dose of cisplatin or 5-FU were shown in Table 2. The combination index (CI) values were calculated to determine the type of drug interaction based on the median-effect principle of the Chou & Talalay method [21]. The CI values for combination treatments of polycarpol and cisplatin ($IC_{20}$) against HeLa cells at 24, 48, and 72 h exposures indicated additive, antagonistic, and antagonistic effects, respectively. The additive effect at 24 h exposure resulted in dose reductions of 2.04-fold for cisplatin and 5.69-fold for polycarpol. The CI values for combination treatments of polycarpol and 5-FU ($IC_{20}$) against HeLa cells at 48 and 72 h exposures indicated antagonistic effects. The CI values for combination treatments of polycarpol and cisplatin ($IC_{30}$) against HeLa cells at 24, 48, and 72 h exposures indicated additive, antagonistic, and antagonistic effects, respectively. The additive effect at 24 h exposure resulted in dose reductions of 1.53-fold for cisplatin and 9.49-fold for polycarpol. The CI values for combination treatments of polycarpol and 5-FU ($IC_{30}$) against HeLa cells at 48 and 72 h exposures indicated antagonistic effects. The CI values for combination treatments of polycarpol and cisplatin ($IC_{40}$) against HeLa cells at 24, 48, and 72 h exposures indicated antagonistic effects. The CI values for combination treatments of polycarpol and 5-FU ($IC_{40}$) against HeLa cells at 48 and 72 h exposures indicated slight synergistic and antagonistic effects, respectively. The synergistic effect at 48 h exposure resulted in dose reductions of 3.80-fold for 5-FU and 2.14-fold for polycarpol. In summary, these results suggest that polycarpol potentiates anticancer activity of cisplatin at sub-toxic doses of $IC_{20}$ and $IC_{30}$ against HeLa cells for 24 h exposure and enhances anticancer activity of 5-FU at a sub-toxic dose of $IC_{40}$ for 48 h exposure against HeLa cells.

To illustrate the drug interactions calculated by the Chou & Talalay method as shown in Table 2, we performed combination treatments of polycarpol at $IC_{50}$ doses derived from the combination treatments (Table 2) with sub-toxic doses ($IC_{20}$, $IC_{30}$, $IC_{40}$) of cisplatin or 5-FU. Drug combination treatments of polycarpol and 5-FU or cisplatin with a sub-toxic dose $IC_{20}$ against HeLa cells were demonstrated in Fig. 2 comparatively with the single drug treatments. Combination treatments of polycarpol and cisplatin signif-

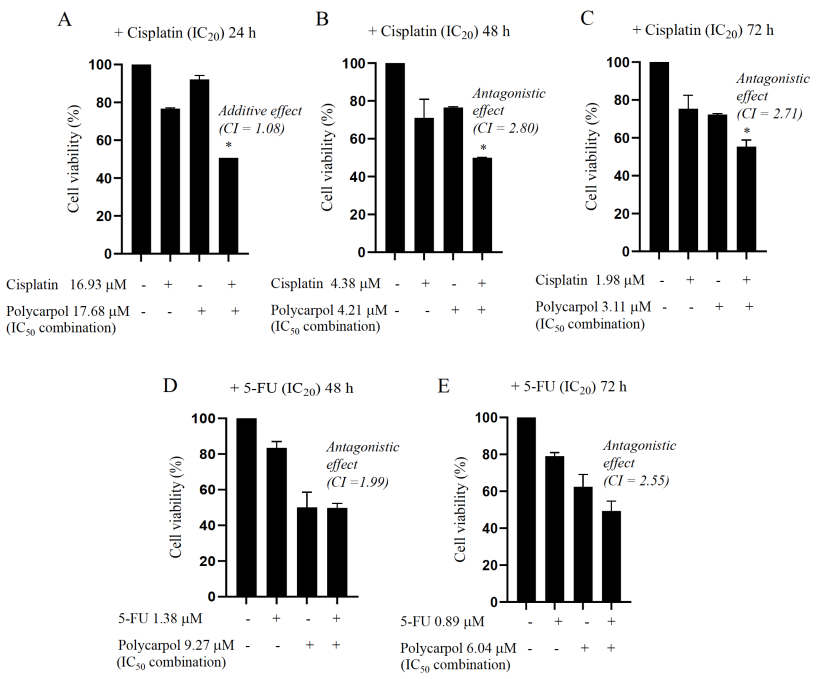


**Fig. 2** Antiproliferative effects of the combination treatment between polycarpol and cisplatin or 5-FU. HeLa cells were treated with polycarpol at $IC_{50}$ concentration of the combination treatment and cisplatin or 5-FU at a sub-toxic dose $IC_{20}$. Cell viability was calculated as a percentage in comparison to cells treated with solvent control (0.50% ethanol + 0.50% DMSO). "*" denotes a statistically significant reduction ($p < 0.05$) compared to the single drug treatment of polycarpol.

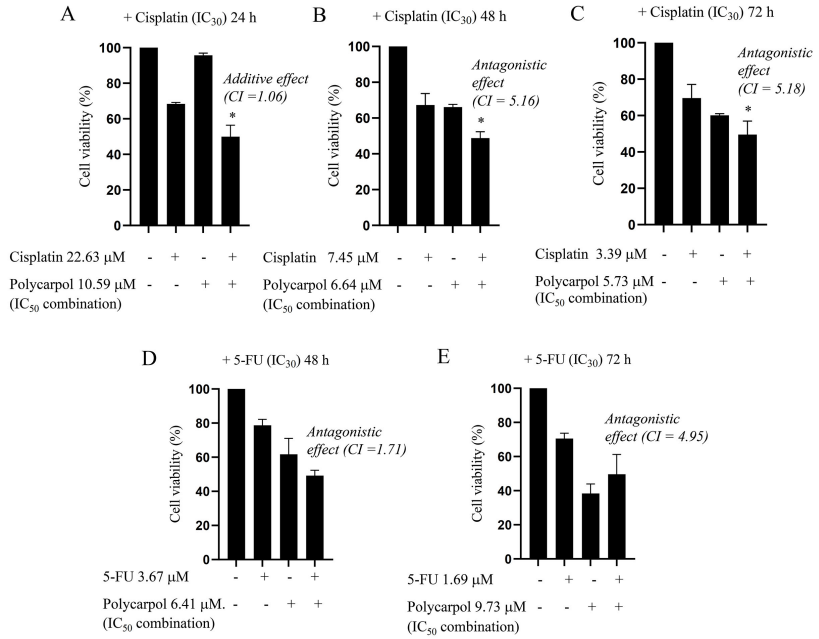


**Fig. 3** Antiproliferative effects of the combination treatment between polycarpol and cisplatin or 5-FU. HeLa cells were treated with polycarpol at $IC_{50}$ concentration of the combination treatment and cisplatin or 5-FU at a sub-toxic dose $IC_{30}$. Cell viability was calculated as a percentage in comparison to cells treated with solvent control (0.50% ethanol + 0.50% DMSO). "*" denotes a statistically significant reduction ($p < 0.05$) compared to the single drug treatment of polycarpol.

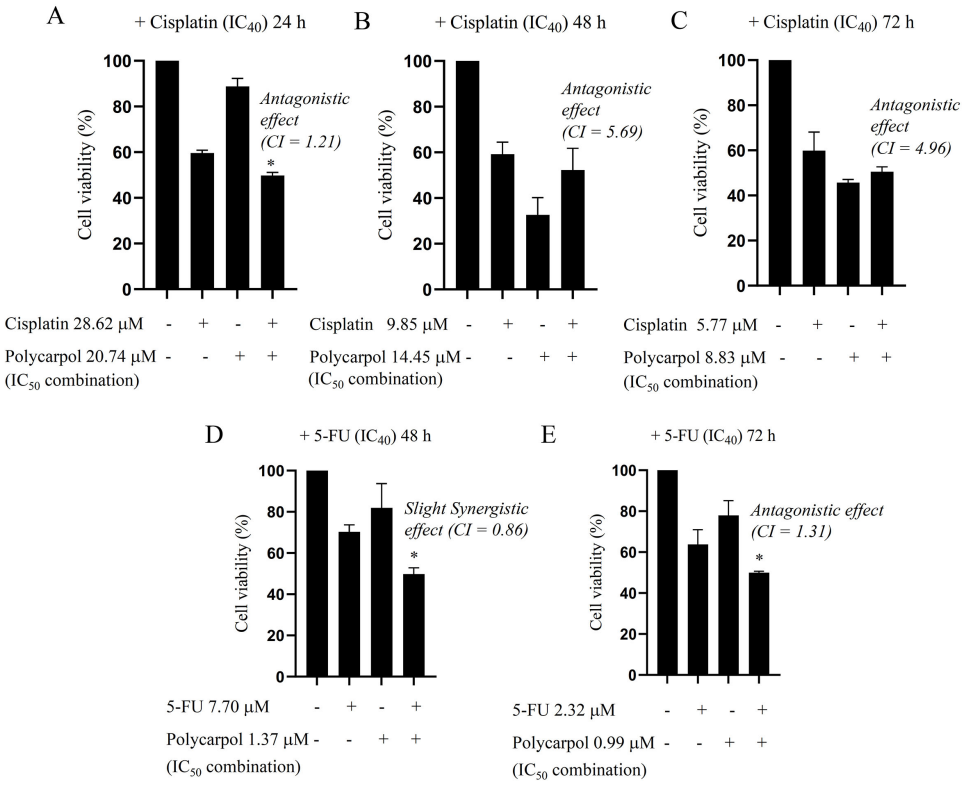


**Fig. 4** Antiproliferative effects of the combination treatment between polycarpol and cisplatin or 5-FU. HeLa cells were treated with polycarpol at $IC_{50}$ concentration of the combination treatment and cisplatin or 5-FU at a sub-toxic dose $IC_{40}$. Cell viability was calculated as a percentage in comparison to cells treated with solvent control (0.50% ethanol + 0.50% DMSO). "*" denotes a statistically significant reduction ($p < 0.05$) compared to the single drug treatment of polycarpol.

icantly reduced proliferation of HeLa cells compared with a single drug treatment of polycarpol (Fig. 2A–C). However, drug interaction showed additive effect only at 24 h exposure (Fig. 2A). Combination treatments of polycarpol and 5-FU did not significantly reduce proliferation of HeLa cells compared with polycarpol single drug treatments (Fig. 2D,E), consistent with their antagonistic effects (Table 2).

Drug combination treatments of polycarpol and cisplatin or 5-FU with a sub-toxic dose $IC_{30}$ against HeLa cells were demonstrated in Fig. 3 comparatively with the single drug treatments. Combination treatments of polycarpol and cisplatin significantly reduced proliferation of HeLa cells compared with a single drug treatment of polycarpol (Fig. 3A–C). However, based on the drug interactions calculated by using the Chou & Talalay method (Table 2), the drug interactions showed additive effect at 24 h exposure (Fig. 3A) but antagonistic effects at 48 h (Fig. 3B) and 72 h (Fig. 3C) exposures. Combination treatments of polycarpol and 5-FU did not significantly reduce proliferation of HeLa cells compared with polycarpol single drug treatments (Fig. 3D,E), consistent with their antagonistic effects (Table 2).

Drug combination treatments of polycarpol and cisplatin or 5-FU with a sub-toxic dose $IC_{40}$ against HeLa cells were demonstrated in Fig. 4 comparatively with the single drug treatments. Combination treatments of polycarpol and cisplatin significantly reduced proliferation of HeLa cells at 24 h exposure (Fig. 4A) but failed to reduce proliferation of HeLa cells at 48 h (Fig. 4B) and 72 h (Fig. 4C) exposures compared with polycarpol single drug treatments. However, the drug interactions calculated by using the Chou & Talalay method (Table 2) showed antagonistic effects at all exposure times. Combination treatments of polycarpol and 5-FU significantly reduced proliferation of HeLa cells at both 48 and 72 h exposures (Fig. 4D,E) compared with polycarpol single drug treatments. However, the drug interaction showed a slight synergistic effect at only 48 h exposure.

## DISCUSSION

Chemotherapy drug toxicity and resistance in cervical cancer remain the major problems to effective practical therapy. These problems need to be dealt with as soon as possible. Therefore, it is important to identify pharmacologically safe agents capable of enhancing

conventional therapy while reducing side effects from chemotherapy drugs. This study highlights the potential of polycarpol from roots of *M. fruticosum* on dose- and time-dependent cytotoxic effects against cervical cancer HeLa cells. Polycarpol potentiated antiproliferative activity of cisplatin at sub-toxic doses $IC_{20}$ and $IC_{30}$ after 24 h exposure with an additive effect against HeLa cells. Moreover, polycarpol enhanced antiproliferative activity of 5-FU at sub-toxic dose $IC_{40}$ after 48 h exposure with a slight synergistic effect against HeLa cells. However, further study is necessary to investigate the molecular mechanism(s) that underlies cytotoxic activity of polycarpol. The cytotoxic activity of polycarpol against cancer cell lines probably involves the inhibition of some enzymes in the cells as demonstrated by other triterpenoid derivatives [22]. Triterpenoids isolated from the fruiting bodies of *Antrodia camphorata* demonstrated cytotoxic activity against several cancer cell lines including a cervical cancer HeLa cell line [23]. Moreover, triterpenoids increase the reactive oxygen species (ROS) generation and inhibit the expression of histone deacetylases (HDACs) [24].

Cisplatin and 5-FU have been shown previously to inhibit proliferation of HeLa cells in a dose- and time-dependent manner [25]. In this study, cisplatin and 5-FU exhibited antiproliferative activity less effective than polycarpol against HeLa cells at an exposure time of 48 h (Fig. 1). Cisplatin exhibited the most effective antiproliferative activity at a shorter exposure time (24 h), whereas 5-FU showed the most effective antiproliferative activity against HeLa cells at a longer exposure time (72 h) (Fig. 1). However, a previous study demonstrated that polycarpol isolated from the roots of *M. fruticosum* showed antiproliferative activity against several cancer cell lines including human epidermoid carcinoma (KB cells), human breast adenocarcinoma (MCF-7 cells), and human hepatocellular carcinoma (HepG-2 cells) but showed no activity against HeLa cells [17]. The discrepancy may be due to the differences in the extraction method and purity of the obtained polycarpol. Indeed, polycarpol used in previous study was isolated by dichloromethane ($CH_2Cl_2$) and methanol (MeOH) and purified by vacuum liquid chromatography over silica gel [17], whereas polycarpol compound used in this study was extracted by ethyl acetate (EtOAc). The extraction and purification methods may impact on the efficiency of biological interactions and yield varying concentrations of the compound leading to differing in biological activity [26]. According to the single drug treatment experiments, polycarpol not only effectively inhibited proliferation of HeLa cells but also exhibited a greater selective toxicity than cisplatin and 5-FU against HeLa cells at all studied exposure times (Table 1), suggesting that polycarpol is a promising anticancer agent for the treatment of cervical cancer.

Chemotherapy using single drug not only affects cancer cell growth but also affects normal cell growth such as mouth, hair, blood, or intestinal cells leading to several side effects [26, 27]. Therefore, combination chemotherapy using the drugs with different mechanisms of drug action is a common strategy to reduce drug resistance development and minimize the adverse effects experienced by cancer patients [29, 30]. In this study, we assessed a potential use of polycarpol to enhance antiproliferative activity of current anticancer drugs (cisplatin and 5-FU) against cervical cancer cells. The CI value is used to assess the effect of combined drugs on the cervical cancer HeLa cells, which may be additive (CI = 0.90–1.10), synergistic (CI < 0.90), or antagonistic (CI > 1.10) [20]. The combinations of polycarpol and cisplatin indicated an additive effect at 24 h exposure for sub-toxic doses of cisplatin at $IC_{20}$ and $IC_{30}$, while the combinations of polycarpol and 5-FU indicated a slight synergism at 48 h exposure for a sub-toxic dose of 5-FU at $IC_{40}$ in HeLa cells (Table 2). The additive effect at a sub-toxic dose $IC_{20}$ resulted in dose reductions of 2.04-fold for cisplatin and 5.69-fold for polycarpol, while additive effect at a sub-toxic dose $IC_{30}$ resulted in dose reductions of 1.53-fold for cisplatin and 9.49-fold for polycarpol. The slight synergistic effect of combined polycarpol and 5-FU at 48 h exposure resulted in dose reductions of 3.80-fold for 5-FU and 2.14-fold for polycarpol. These results suggest that polycarpol exhibits a promising drug combination to potentiate anticancer activity of cisplatin and to enhance anticancer activity of 5-FU for the treatments of cervical cancer.

The potential role of polycarpol in the management of cancer treatment against human cervical cancer cells has not yet been elucidated. However, its cytotoxic activity against cancer cell lines is probably due to the inhibition of some enzymes like the other triterpenoid derivatives [22, 31]. Triterpenes demonstrated cytotoxic activity against human colon cancer cells by inducing apoptosis through the expression suppression of the anti-apoptotic protein Bcl-2 [31]. The triterpene Antcin-H, isolated from the mushroom *Antrodia cinnamomea*, suppressed the growth of renal cancer cells through suppression of FAK-related signaling pathway (Src, FAK, paxillin, and ERK1/2), inhibition of focal adhesion turnover and lamellipodium formation, inactivation of c-Fos and C/EBP-β, down-regulation of MMPs (especially MMP-7), and up-regulation of TIMP (TIMP-3 and TIMP-4) expressions [32]. In addition, the triterpenoid (3β,7β,25-trihydroxycucurbita-5,23(E)-dien-19-al, TCD) isolated from wild bitter gourd showed antiproliferative activity against breast cancer cells (MCF-7 and MDA-MB-231 cells) [24]. TCD induced apoptosis through down-regulation of Akt-NF-κB signaling, up-regulation of p38 mitogen-activated protein kinase and p53, generation of reactive oxygen species, and inhibition of histone deacetylase (HDAC) expression.

## CONCLUSION

Polycarpol exhibited antiproliferative activity against cervical cancer HeLa cells with a dose- and time-dependent manner. Polycarpol exhibited remarkable cytotoxic effect towards HeLa cells at 48 h exposure compared with 5-FU and cisplatin. The polycarpol combination treatments showed synergistic effect with 5-FU ($IC_{40}$) at 48 h exposure and additive effects with cisplatin ($IC_{20}$ and $IC_{30}$) at 24 h exposure. The findings of this study suggest that polycarpol potentiates the antiproliferative activity of cisplatin and enhances the antiproliferative activity of 5-FU against HeLa cells. Polycarpol could potentially be an alternative therapeutic agent for the treatment of cervical cancer. However, further studies on the mechanisms of drug action and anticancer activity in animal models are required.

## Appendix A. Supplementary data

Supplementary data associated with this article can be found at https://dx.doi.org/10.2306/scienceasia1513-1874.2025.025.

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dimethylchalcone from *Syzygium nervosum* seeds on antiproliferative, DNA damage, cell cycle arrest, and apoptosis in human cervical cancer cell lines. *Molecules* **27**, 1154.

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## Appendix A. Supplementary data

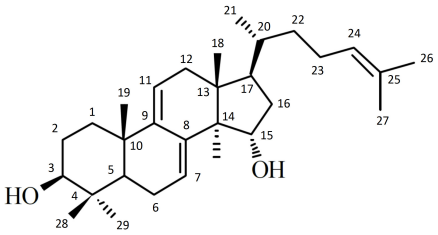


**Fig. S1** Chemical structure of polycarpol (MW = 440.7 Dalton).

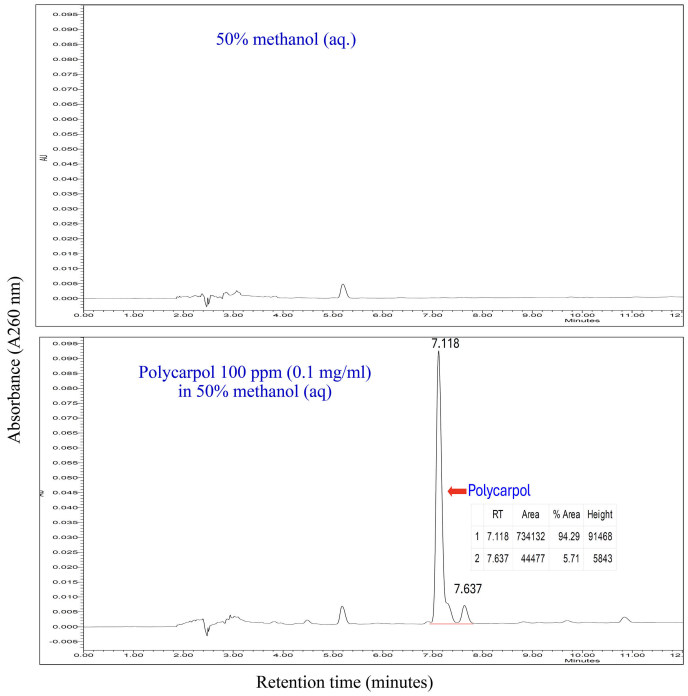


**Fig. S2** HPLC profile of polycarpol.

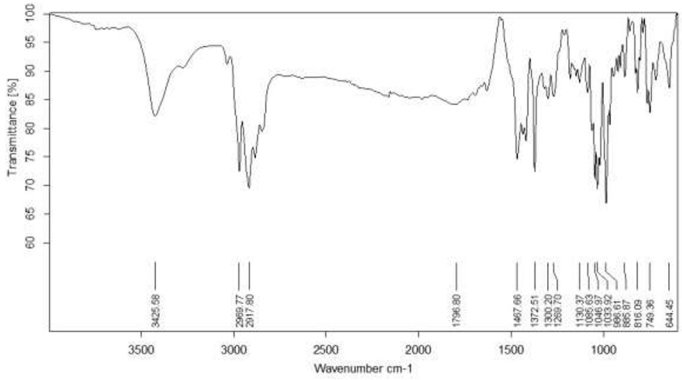


**Fig. S3** IR spectrum of polycarpol.

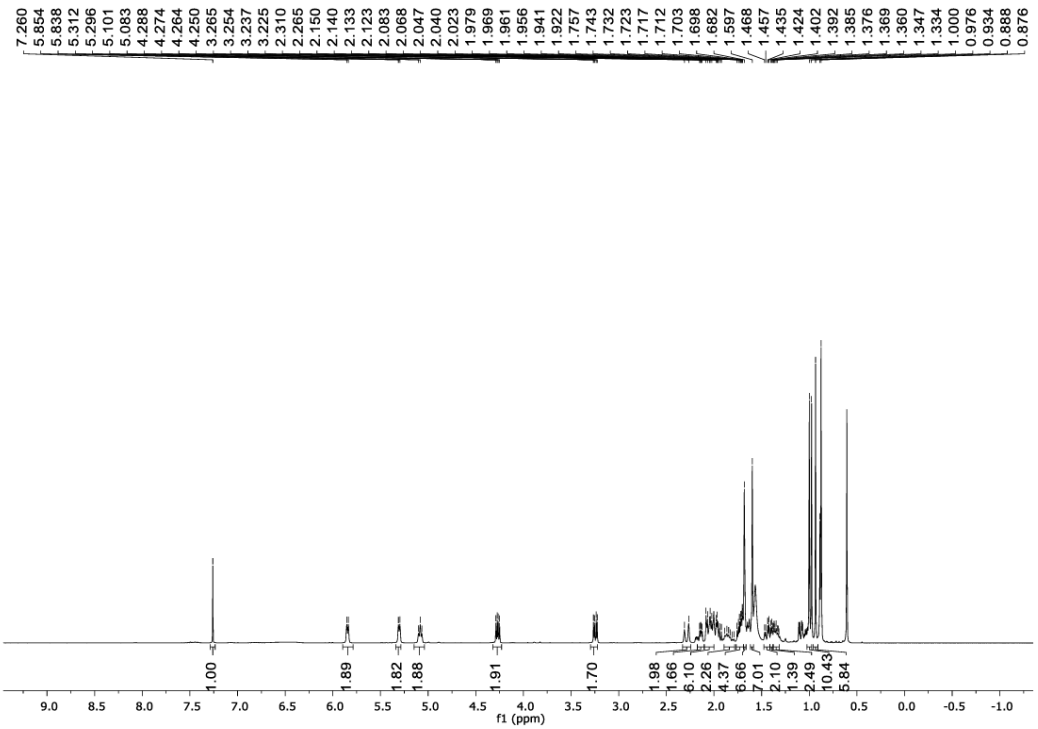


**Fig. S4** $^{1}$H NMR spectrum of polycarpol in $CDCl_3$.

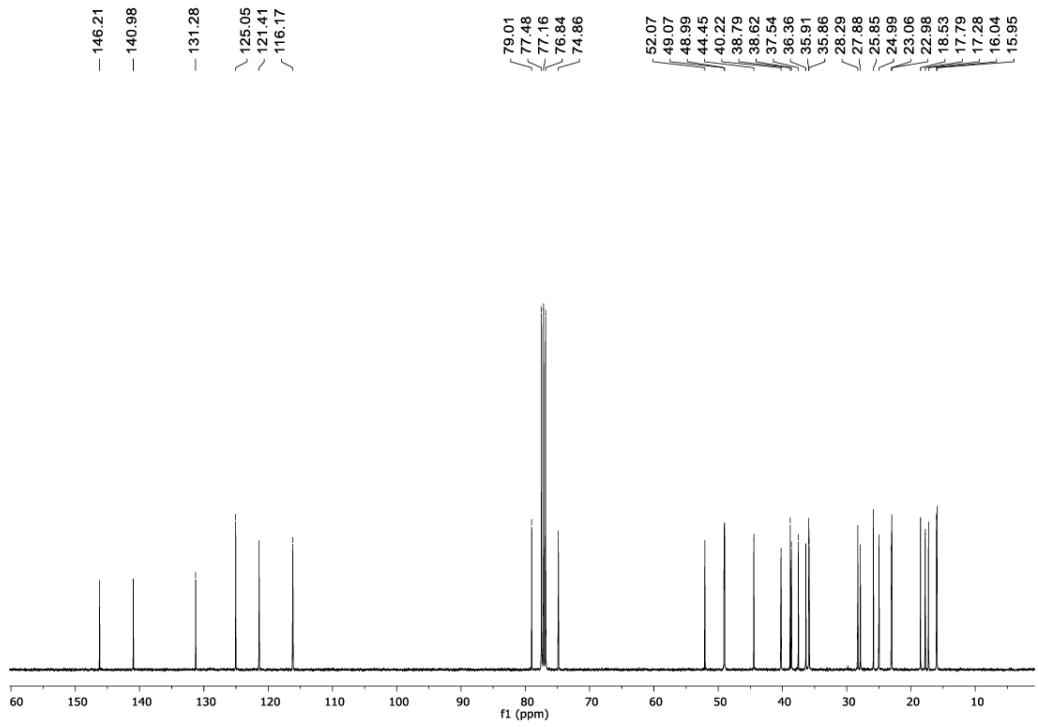


**Fig. S5** $^{13}$C NMR spectrum of polycarpol in $CDCl_3$.

**Table S1** $^{1}$H (400 MHz) and $^{13}$C NMR (100 MHz) data of polycarpol ($CDCl_3$)

| No. | $\delta_H$ (J in Hz) | $\delta_C$ |
|---|---|---|
| 1 | 1.98, m<br>1.40, m | 35.9 |
| 2 | 1.71, m<br>1.65, m | 27.9 |
| 3 | 3.25, dd (11.2, 6.8) | 79.0 |
| 4 | | 38.9 |
| 5 | 1.45, dd (13.2, 8.8) | 49.1 |
| 6 | 214, m<br>2.05, m | 23.0 |
| 7 | 5.85, d (6.4) | 121.4 |
| 8 | | 141.0 |
| 9 | | 146.2 |
| 10 | 37.5 | |
| 11 | 5.30, d (6.4) | 116.2 |
| 12 | 2.29, d (18.0)<br>2.04, m | 38.6 |
| 13 | | 44.5 |
| 14 | | 52.1 |
| 15 | 4.27, m | 74.9 |
| 16 | 1.96, m<br>1.72, m | 40.0 |
| 17 | 1.30, m | 49.0 |
| 18 | 0.61, s | 16.0 |
| 19 | 0.93, s | 23.1 |
| 20 | 1.85, m | 36.4 |
| 21 | 0.89, s | 18.5 |
| 22 | 1.34, m<br>1.03, m | 35.9 |
| 23 | 1.97, m | 25.0 |
| 24 | 5.08, t (7.2) | 125.1 |
| 25 | | 131.3 |
| 26 | 1.60, s | 25.9 |
| 27 | 1.68, s | 17.8 |
| 28 | 0.88, s | 17.3 |
| 29 | 0.98, s | 28.3 |
| 30 | 1.00, s | 17.3 |

Polycarpol: needle: $[\alpha]D^{25} = +102.0$, $c = 1.00$ in $CHCl_3$): IR (neat) $\nu_{max}$ 3426, 2970, 2918, 1797, 1468, 1373, 1300, 1270, 1130, 1086, 1047, 1034, 987, 886, 816, 749, and 645 cm$^{-1}$:@ $^{1}$H NMR (400 MHz); $\delta = 5.85$ (d, $J = 6.4$Hz, 1H, H-7), 5.30 (d, $J = 6.4$ Hz, 1H, H-11), 5.08 (t, $J = 7.2$ Hz 1H, H-24), 4.27 (m, 1H, H-15), 3.25 (dd, $J = 11.2$, 6.8 Hz, 1H, H-3), 2.29 (d, $J = 18.0$ Hz, 1H, H-12a), 2.14 (m, 1H, H-6a), 2.05 (m, 1H, H-6b), 2.04 (m, 1H, H-12b), 1.98 (m, 1H, H-1a), 1.97 (m, 1H, H-23), 1.96 (m, 1H, H-16a), 1.85 (m, 1H, H-20), 1.72 (m, 1H, H-16b), 1.71 (m, 1H, H-2a), 1.68 (s, 1H, H-27), 1.65 (m, 1H, H-2b), 1.60 (s, 1H, H-26), 1.45 (dd, $J = 13.2$, 8.8 Hz, H-5), 1.40 (m, 1H, H-1b), 1.34 (m, 1H, H-22a), 1.30 (m, 1H, H-17), 1.00 (s, 1H, H-30), 1.03 (m, 1H, H-22b), 0.98 (s, 1H ,H-29), 0.93 (s, 1H, H-19), 0.89 (s, 1H, H-21), 0.88 (s, 1H, H-28), and 0.61 (s, 1H, H-18): $^{13}$C NMR (100 MHz); $\delta = 146.2$ (C-9), 141.0 (C-8), 131.3 (C-25), 125.1 (C-24), 121.4 (C-7), 116.2 (C-11), 79.0 (C-3), 74.9 (C-15), 52.1 (C-14), 49.1 (C-5), 49.0 (C-17), 44.5 (C-13), 44.0 (C-16), 38.9 (C-4), 38.6 (C-12), 37.5 (C-10), 36.4 (C-20), 35.9 (C-1), 35.9 (C-22), 28.3 (C-29), 27.9 (C-2), 25.9 (C-26), 25.0 (C-23), 23.1 (C-19), 23.0 (C-6), 18.5 (C-21), 17.8 (C-27), 17.5 (C-28), 16.0 (C-18), and 16.0 (C-30).