

# Quercetin and kaempherol: an argument against the use of podophyllin?

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## Abstract

**Introduction**—Topical application of podophyllin is a routine procedure in patients with ano-genital warts. Podophyllin is a crude plant extract and is therefore not a well-defined product. It may contain variable amounts of the active lignan podophyllotoxin and the majority of the dry weight of podophyllin is made up of substances never identified.

**Objective**—The purpose of the study was to estimate in podophyllin 20% the amounts of two mutagenic substances, quercetin and kaempherol.

**Methods**—Using high-pressure liquid chromatography the amounts of quercetin and kaempherol were determined in 3 batches of podophyllin 20%.

**Results**—Quercetin and kaempherol constitutes 2.5–3.8% and 6.0–6.4% of podophyllin dry substance, respectively. Podophyllotoxin constitutes in comparison 12.7–13.8% of podophyllin dry substance.

**Conclusion**—As approximately 10% of the amount of dry substance in podophyllin 20% is composed of two mutagenic flavonoids, quercetin and kaempherol, efforts should be focused on the production of a well-defined purified podophyllotoxin preparation that may replace podophyllin for clinic use in patients with genital warts. Self-medication with purified podophyllotoxin 0.5% may be considered as first-line treatment in well-instructed patients with external genital warts.

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clinical trials to be effective and safe in the licensed indications. However, since the 1940s a routine treatment for condyloma acuminatum has been topically applications of 20–25% podophyllin in ethanol, a crude mixture of cytotoxic materials from plant resins originating from either of two podophyllum species: *Podophyllum peltatum* and *Podophyllum emodi*.<sup>1</sup> The most important ingredient of podophyllin, quantitatively and therapeutically, is podophyllotoxin.<sup>2,3</sup> Podophyllin contains in addition a number of other lignans including alpha-peltatin, beta-peltatin and 4-dime-thylpodophyllotoxin.<sup>4,5</sup>

Part of the color of podophyllin is due to the presence of a flavonoid-pigment called quercetin.<sup>6,7</sup> Quercetin (3,3', 4', 5,7-pentahydroxy-flavone) occurring in conjugated forms in many plant products has been shown to be a highly mutagenic substance.<sup>8</sup> In order further to characterize podophyllin we quantitatively estimated the content of quercetin and another mutagenic flavonoid kaempherol (3,4',5,7-tetrahydroxyflavone)<sup>9</sup> in three batches of podophyllin 20%.

## Methods

The analyses were carried out by means of a high-pressure liquid chromatography (HPLC)—system consisting of Shimadzu LC-6A liquid chromatograph, SPD-6AV UV-VIS detector, SCL-6B system controller and an C-R5A integrator. The column was a steel column with Spherisorb S3 ODS 1 (100 × 4.6 mm diameter) and the mobile phase consisted of methanol, H<sub>2</sub>O and phosphoric acid in the ratio 450:545:5. The UV detection was performed at 370 nm. As standards quercetin R8, quercetin dihydrate (Art. 17196-4 Aldrich), kaempherol RS, kaempherol (Art. 60010, Fluka) were used.

## Results

In the table it can be seen that quercetin composes 2.5–3.8% of the dry substance of podophyllin 20%.

## Introduction

It is widely accepted that drugs used in human medicine should ideally contain well-defined active substances shown in controlled

The content of two mutagenic flavonoids, quercetin and kaempherol, determined in 3 batches of podophyllin 20%

	Podophyllin 20%					
	Batch No 1		Batch No 2		Batch No 3	
	Percentage of weight of solution	Percentage of podophyllin dry substance	Percentage of weight of solution	Percentage of podophyllin dry substance	Percentage of weight of solution	Percentage of podophyllin dry substance
Quercetin	0.5	2.5	0.8	3.8	0.6	3.0
Kaempherol	1.2	6.0	1.3	6.4	1.3	6.3
Podophyllotoxin	2.8	13.8	2.6	12.8	2.6	12.7
Dry substance	20.1	100.0	20.5	100.0	20.0	100.0

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phyllin. Kaempferol makes up about 6% of the dry substance of podophyllin. About 13% of the amount of dry substance was identified as podophyllotoxin. Two batches of podophyllin (no. 1–2, table) had nearly identical amounts of podophyllotoxin, whereas batch no. 3 had a 10% higher concentration of the active principle.

### Discussion

This study has documented that approximately 10% of the amount of dry substance in podophyllin is made up of the two very mutagenic flavonols, namely quercetin and kaempferol. Podophyllotoxin accounts for about 13% of the weight, but the major part of the remaining 75% of the dry substance is made up by decomposition products of podophyllotoxin, peltatins and substances which have never been identified.

Quercetin has previously been extracted from podophyllin by simplified methods with yields of 2.4% (American podophyllin)<sup>7</sup> and 1.3% (Indian podophyllin).<sup>10</sup> Kaempferol has to our knowledge never previously been isolated from podophyllin.

Although quercetin is mutagenic in bacteria and insects and caused gene conversion in yeast and chromosomal anomalies in cultured cells<sup>8</sup> equivocal results were obtained in animal carcinogenicity studies.<sup>8</sup> Kaempferol has been shown to be mutagenic in bacteria and insects and in mammalian cells *in vitro*.<sup>9</sup> It also induces micronuclei in mice.<sup>9</sup> Quercetin and kaempferol occurs in plants as various glycosides which are not mutagenic. These may, however, hydrolyse to mutagenic aglycones by beta-glycosidases present in the mammalian intestinal flora.<sup>11</sup> The suspected contribution of flavonoids to the induction of cancer is supported by the fact that cattle infected with bovine papillomavirus develop lung cancer after feeding with fern containing large amounts of quercetin.<sup>12</sup> An epidemiological study shows a direct correlation between the incidence of cancer of the stomach in different parts of Japan and the content of quercetin and rhamnetin in local types of pickles.<sup>13</sup>

Infection with human papillomavirus (HPV) is known to be associated with genital neoplasia<sup>14</sup> and treatment of HPV-induced lesions with a potentially mutagenic substance, such as podophyllin, may be a matter of concern. Treatment of genital warts with podophyllin can induce histologic changes including increase in the number of mitotic figures and necrosis in warts tissue.<sup>15</sup> These changes in no way simulate those of squamous cell carcinoma.<sup>15</sup> It should therefore be emphasised that at present there are no data to support that podophyllin should act as a co-carcinogen in HIV-infected patients.

An alternative to podophyllin is self-application of a purified podophyllotoxin 0.5% solution (Condyline, Condylox).<sup>16</sup> Podophyllotoxin can be used in both women and men with externally located genital warts.<sup>16,17</sup>

This topical preparation does not contain flavonoids and in the AMES test for mutagenesis, podophyllotoxin was found to be a non-mutagen.<sup>18</sup> In a mitotic chromosomal aberration test podophyllotoxin induced micronuclei in the bone marrow of mice (personal communication, Nycomed-DAK, Denmark).<sup>11</sup> This is not surprising as it is known that podophyllotoxin causes mitotic arrest, which is the basis of its therapeutic activity in genital warts. In addition prolonged topical application of podophyllotoxin is without carcinogenic activity in mice.<sup>19</sup>

To replace podophyllin another approach could be the use of purified higher-concentrated solutions of podophyllotoxin. A 5% podophyllotoxin solution applied once weekly has been shown to be as effective and safe as podophyllin 20% in a controlled clinical trial in patients with genital warts.<sup>20</sup> If a high-concentrated purified podophyllotoxin solution was developed and licensed it could with advantage replace the marketed podophyllin.

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