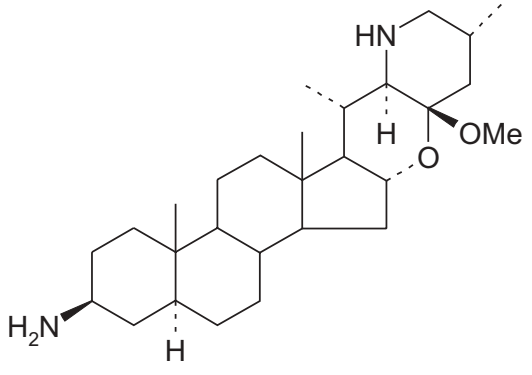
In vitro cytotoxic properties of O-methylsolanocapsine isolated from *Solanum pseudocapsicum* leaves

Solanum pseudocapsicum (family-Solanaceae) is an erect, much branched, nonspiny bushy shrub distributed in the gardens of Simla, Mussoorie, the Doon valley, the Nilgiris, and other high-altitude places in India. Its antimicrobial, antiviral, antispasmodic, antihypertensive, antioxidant, and hepatoprotective properties are well known. The 50% methanol extract of the entire plant of *Solanum pseudocapsicum* has also been reported to possess cytotoxic activity in a preliminary study by Dhar *et al*.[1] Earlier studies in our laboratories have shown significant *in vitro* cytotoxic and *in vivo* antitumor properties of the total alkaloid fraction of *Solanum pseudocapsicum*.[2,3] The total alkaloid fraction of the leaves was found to be more potent. Several plants belonging to the genus exhibited strong cytotoxic and antitumor properties. *Solanum trilobatum, Solanum incanum, Solanum capsicastrum, Solanum gnera, Solanum indicum, Solanum sodomaeum*, and *Solanum nigrum* are a few plants reported to have potential antitumor activities.[2] Hence, based on these studies we were interested in isolating the active constituents from the total alkaloid fraction of *Solanum pseudocapsicum* leaves. Several steroidal alkaloids including solanocapsine, solacasine, solacapine, episolacapine, isosolacapine, and O-methylsolanocapsine were isolated from the arboreal part of the plant.[4] The present study describes the isolation of O-methylsolanocapsine [Figure 1] and its *in vitro* cytotoxic properties against several cancerous and Vero normal cell lines.

The leaves of *Solanum pseudocapsicum* (Voucher No 7345) were collected during July 2003 from the Government Arts College grounds, Ootacamund, Tamil Nadu and authenticated by Medicinal Plants Collection Unit, Government Arts College, Ootacamund. The fresh leaves were shade dried, powdered (190 gm), and extracted with methanol (700 ml) in a Soxhlet extractor for 18-20 h. The extract was concentrated under reduced pressure and controlled temperature to yield a deep brown semisolid residue (48.2 gm, 25.37% w/w). The total alkaloid fraction was isolated from the extract of the leaves (0.192%) following a conventional procedure.[2]

Figure 1: Structure of O-methylsolanocapsine



The crude alkaloid mixture (4 gm) was chromatographed on a neutral alumina-packed column and eluted with gradients of petroleum ether, chloroform, ethyl acetate, and methanol to afford a number of fractions. Successive elutions with 10-20% chloroform in petroleum ether afforded a white precipitate, which was recrystallized from the chloroform: acetone mixture and identified as O-methylsolanocapsine (0.5%), m. p. 183°C. ^{1}H NMR (MEOD): δ 0.73 (s, 3H, 10-Me), δ 0.77 (s, 3H,13-Me), δ 0.84 (d, 3H, 25-Me), δ 0.98 (d, 3H, 20-Me), δ 3.02 (dd, 1H, 26-H), δ 3.10 (s, 3H, OMe), δ 4.54 (m, 1H, 16-H).[4,5]

The Vero, HEp-2, HeLa, and A-549 cell cultures used in these experiments were obtained from the National Center for Cell Sciences, Pune, and Pasteur Institute of India, Coonoor. Stock cells of HEp-2, RD, and Vero cell lines were cultured in RPMI-1640 and DMEM, supplemented with 10% inactivated sheep serum, penicillin (100 IU/ml), streptomycin (100 μg/ml), and amphotericin B (5 μg/ml) in a humidified atmosphere of 5% CO2 at 37°C until confluent. The cells were dissociated with 0.2% trypsin and 0.02% EDTA in PBS. The stock cultures were grown in 110 ml flat bottles and all experiments were carried out in 96-well microtiter plates, where the cell population was adjusted to 10,000 cells per well.

Cell lines in exponential growth phase were washed, trypsinized, and resuspended in complete culture media. Cells were plated at 10,000 cells/well in 96-well microtitre plates and incubated for 24 h, during which a partial monolayer formed. The cells were then exposed to various concentrations (10-1000 μg/ml) of O-methylsolanocapsine in quadruplicate. Control cells received only maintenance medium. The plates were incubated at 37°C in a humidified incubator with 5% CO_2 for a period of 72 h. Morphological changes of the sample treated cells were examined using an inverted microscope at different time intervals and compared with the cells serving as control. At the end of 72 h, cellular viability was determined by using MTT and SRB assays,[6,7] and the IC_{50} value (concentration of the sample required to kill 50% of the cells) was calculated.

The ^{1}H NMR spectrum showed three protons doublet at δ 0.98 and 0.84 assigned, respectively, to 20-Me and 25-Me protons, in analogy with spirostane sapogenins. It exhibited two singlets at δ 0.73 and 0.77 for three protons each for 10-Me and

13-Me groups, at δ 4.54 for an oxymethine protons (H-16), at δ 3.02 for two protons for methylene (H-26), and three protons singlet at δ 3.10 assignable to an O-CH_3 group. ^{13}C NMR values were comparable with the values of O-methylsolanocapsine reported in literature.[4,5] From this study, it was confirmed that O-methylsolanocapsine is more cytotoxic to the HeLa cell lines, with IC_{50} values of 39.90 ± 0.03 and 34.65 ± 0.06 by MTT and SRB assays, respectively [Table 1].

Reports indicate that steroidal alkaloids from the *Solanum* species possess strong cytotoxic and antitumor properties.[1-3] Solamargine from *Solanum nigrum*, incanumine from *Solanum sodamaeum,* solasonine from *Solanum crinitum,* and several other steroidal alkaloidal glycosides are known to possess these properties.[2] The present study indicates that O-methylsolanocapsine isolated from *Solanum pseudocapsicum* leaves also possess strong cytotoxic properties. This merits further investigation in *in vivo* models to confirm its antitumor activity. However, in our earlier studies[2] the total alkaloid fraction of the methanol extract exhibited more potent cytotoxicity against these cell lines. Hence, the other alkaloids present in the total alkaloid fraction or the total mixture of alkaloids may be responsible for the high cytotoxic effect of the fraction. Several studies have indicated more potent activity of crude extracts compared to their isolated active compounds used alone.[8] Similar results were observed by us. Further studies are needed to isolate other alkaloids from the total alkaloid fraction and screen them for cytotoxic properties.

Table 1

Cytotoxic activity of O-methylsolanocapsine by MTT and SRB assays

Cell lines	*IC_{50} ± S. E. of O-methylsolanocapsine by*	
	MTT	*SRB*
HeLa	39.90 ± 0.03	34.65 ± 0.06
HEp-2	46.60 ± 0.07	50.40 ± 0.02
A-549	64.96 ± 0.01	57.80 ± 0.08
Vero	59.74 ± 0.03	53.68 ± 0.02

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