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PLANT ANTITUMOR AGENTS, 24.[1] RAPID 9-KB ASSAY

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The standard 9-KB cyctotoxicity assay that utilizes cells derived from a human epidermoid carcinoma of the nasopharynx was initially described by Eagle (1,2), was standardized by the National Cancer Institute (3), and has been useful for many years as a preliminary screen for cytotoxicity or for fractionating plant samples prior to carrying out assays for in vivo activity.

We had occasion, however, to screen more than 1600 plant samples for 9-KB in a limited time period with a limited staff. We wish to describe a rapid, simplified 9-KB assay designed to test for activity at a cut-off point of ED_{50}, 20 μg/ml, which not only increased our weekly productivity from 40 to 200 samples but also reconfirmed most of the samples found active at 20μg/ml when retested by the standard procedure (3).

EXPERIMENTAL

The 9-KB cells are brought into an active growth phase exactly as described in standard procedure (3). The sample to be assayed (in our case CH_2Cl_2 extracts prepared from EtOH extracts) was dissolved in the solvent of choice (we usually used 95% EtOH or H_2O in the case of aqueous fractions) to give a concentration of 20 mg/ml and then diluted to 80 μg/ml with media containing the 9-KB cells. Only one dilution is assayed per sample in contrast to the standard procedure that utilizes three or more dilutions.

After incubation at 37° for 72 h (3), the tubes were removed and visually examined for color. Samples with heavy cell growth (no inhibition at 20 μg/ml) will be yellow; those with low growth will be red, indicating inhibition at 20 μg/ml.

As a check, media can be removed from the tubes, the 9-KB cells washed with saline, drained, and Lowry's reagent added. After heating for 5 min at 60°, 0.5 ml of Folin's reagent is added and the optical density (OD) determined using a suitable photometer set at a wavelength of 660 nm (3). This OD can be compared with the value obtained from cells grown in the absence of any inhibitor.

RESULTS AND DISCUSSION

Using the simplified procedure we screened 1628 plant extracts at a rate of 200/week compared with 40 by the standard procedure. The method required less than 3 days/week of technician time compared with 5 days/week in the standard procedure. The procedures are compared in Table 1.

TABLE 1. Comparison of Standard and Rapid 9-KB Procedures

Procedure	Number of Sample Dilutions Tested	Total Tubes	Total Volume Growth Media (ml)	Incubation Time (h)	Cell Digestion	Folin Reagent	Reading[a] Method	Calculations
Standard	5	10	40	72	yes	yes	optical density	yes
Rapid	1	1	4	72	no	no	visual	no

[a]If visual color changes should be questionable, OD readings can be made as in the standard procedure.

Of the 1628 samples tested, 59 showed activity at the 20 μg/ml level. Of these, 40 were active at 10 μg/ml or lower when reassayed by the standard method (3). Table 2 gives the names and activity of the cytotoxic samples that confirmed at ED_{50}, 1×10^1 ml. The 20 μg/ml ED_{50} value adopted as a cut off point is reasonable based on our extensive experience over a 25-year period in comparing in vitro 9 KB and in vivo P-388 assay data. We have rarely obtained other than negative or marginal P-388 activity in extracts which showed ED_{50} in 9 KB>20 μg/ml. The above proce-

[1]For Part 23 in this series see M.C. Wani, A.W. Nicholas, and M.E. Wall, *J. Med. Chem.*, **29**, 2358 (1986).

TABLE 2. 9-KB Activities[a]

Family	Genus	Species	Plant Part[b]	Source	9-KB Activity
Apocynaceae	*Roupellina*	*boivin*	st,lf,fl	Madagascar	3×10^{0}
Aristolochiaceae	*Aristolochia*	*debilis*	st,ws,sb	China	5×10^{0}
Asteraceae	*Carpesium*	*abrotanoides*	st,lf	China	5×10^{0}
	Olearia	*calcarea*	rt,st,lf,fl,fr	W. Aust.	5×10^{0}
	Sonchus	sp.	rt,st,lf,fl	W. Aust.	5×10^{0}
Bignoniaceae	*Markhamia*	*platycalyx*	rt	India	7×10^{0}
Boraginaceae	*Lithospermum*	*erythrorhizon*	rt	China	5×10^{-1}
Celastraceae	*Psammamoya*	*choretroides*	rt,st,lf,fl	W. Aust.	5×10^{0}
Cucurbitaceae	*Bolbostemma*	*paniculatum*	rt	China	3×10^{0}
	Hemsleya	*macrosperma*	rt	China	9×10^{0}
Epacridaceae	*Andersonia*	*parvifolia*	rt,st,lf,fl	W. Aust.	5×10^{0}
Fabaceae	*Oxylobium*	*parviflorum*	rt	W. Aust.	1×10^{1}
Haemodoraceae	*Anigozanthes*	*humilis*	rt,st,lf,fl	W. Aust.	5×10^{0}
	Anigozanthes	*mangglesii*	rt,st,lf,fl	W. Aust.	1×10^{1}
Hydnoraceae	*Hydnora*	*escalaenta*	rh	Madagascar	1×10^{1}
Iridaceae	*Homeria*	*miniata*	*bu,st,lf,fl*	*W. Aust.*	5×10^{-1}
Liliaceae	*Asparagus*	*asparagoides*	rt,st,lf,fl	W. Aust.	5×10^{0}
Miliaceae	*Malleastrum*	sp.	rt,st,lf,fl	Madagascar	4×10^{0}
Menispermaceae	*Pericampylus*	*glaucus*	st,lf	China	9×10^{0}
Myrtaceae	*Melaleuca*	*pentagona*	rt	W. Aust.	1×10^{1}
	Verticordia	c.f. *grandifloria*	rt,st,lf,fl	W. Aust.	5×10^{0}
Pinaceae	*Pseudolaria*	*kaempferi*	st,ws,sb	China	7×10^{-1}
Proteaceae	*Banksia*	*laevigata*	fr	W. Aust.	5×10^{0}
	Banksia	*laevigata*	rt	W. Aust.	1×10^{1}
	Banksia	*laeviagata*	ws, sb	W. Aust.	5×10^{0}
	Dryandra	*armata*	st,lf,fl	W. Aust.	5×10^{0}
	Grevillea	*baxteri*	rt	W. Aust.	5×10^{0}
	Grevillea	*excelsior*	rt	W. Aust.	5×10^{0}
	Grevillea	*pilosa*	rt	W. Aust.	1×10^{1}
	Grevillea	*pilosa*	tw,lf,fl	W. Aust.	1×10^{1}
	Grevillea	*pilosa*	ws,sb	W. Aust.	1×10^{1}
	Hakea	*costata*	rt	W. Aust.	5×10^{0}
	Isopogon	*scabriusculus*	rt	W. Aust.	5×10^{0}
	Xylomelum	*angustifolium*	fr	W. Aust.	4×10^{0}
Rhamnaceae	*Pomaderris*	*ovaria*	rt,ws,sb	W. Aust.	1×10^{1}
Rubiaceae	*Rubia*	*cordifolia*	st,ws,sb	China	5×10^{0}
Simaroubaceae	*Brucea*	*javanica*	fr	China	3×10^{0}

[a]Three active samples are not shown because of unconfirmed results.

[b]bu=bulb, fl=flower, fr=fruit, lf=leaf, rh=rhizome, rt=root, sb=stem bark, st=stem, tw=twig, ws=stem wood.

dure can also be used as a rapid screen in fractionation procedures, setting the desired single inhibition concentration to the appropriate level, i.e., 1×10^{-1}, 1×10^{-2} etc., in this way eliminating inactive or less interesting chromatographic fractions.

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