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An Approach to the Study of a Medicinal Plant: Passiflora incarnata L. (***)

At present many medicinal plants have a well demonstrated pharmacological activity, but the structures responsible for this activity are still unknown. It depends on several problems concerning either the chemical structures, or the quantity of the active principles present in the whole plant or the presence of analogous compounds having the same structure but with different pharmacological activity.

In order to overcome these difficulties, a new tool can now be employed: plant cell cultures. It is very well known that plant cell cultures produce, in suitable conditions, almost all the same secondary metabolites produced by the whole original plant and sometimes in higher quantities [1, 2, 3, 4, 5]. Moreover, the extraction of secondary metabolites from plant cell cultures is easier because of the absence of chlorophyls and lipophilic products such as waxes and fats. The absence also of hard tissues such as wood or roots allows the extraction to proceed quickly and under mild conditions.

In addition, the main advantage of cell culture, compared with the whole plant, is that it can be obtained in the desired quantity independently of the biological cycle of the plant itself. In the present work we have compared the pharmacological activity of the Passiflora incarnata L. extract (kindly supplied by Aboca S. Sepolcro) with the extract of cell cultures from the same plant.

Aerial parts of one-year-old P. incarnata, mainly leaves, were extracted as soon as harvested with the same quantity (w/v) of EtOH. After 24 h at room temperature, the mixture was filtered and a first ethanolic extract was obtained (E I). The solid residue after filtration was again extracted with the same volume of pure EtOH and afforded a second ethanolic extract (E II).

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The solvent of the two extracts was evaporated and the aqueous residue was freeze-dried.

Cell cultures of P. incarnata were obtained from shoots or stems grown on solid medium (callus cultures) and then transferred into liquid medium (suspended cultures) with the usual techniques reported in literature [6].

Both kinds of cultures were used in this work. Twenty day old callus cultures were filtered and extracted three times with 80% EtOH. Fifteen day old suspended cultures were filtered too and the cells extracted three times with 80% EtOH. The ethanolic extracts from callus and suspended cells were evaporated under reduced pressure and the resulting aqueous phase was freeze-dried.

Also the solid material from supernatant of suspended cell cultures was freeze-dried tested, but resulted inactive.

The ethanolic extract from plant and cell cultures were evaporated under reduced pressure and the resulting aqueous phase was freeze-dried and their activity was evaluated for pharmacological activity.

Analgesimetric procedures

The nociceptive threshold was evaluated using the tail-flick [7], the hot plate [8] and vocalization [9] tests. In the tail-flick test the strength of the radiant heat was adjusted so as to obtain a tail-flick latency of 3.7 ± 0,15 s in control animals, and values were recorded by an automated device. A cut-off time of 10 s was fixed. For the hot plate test a plate temperature of 53±0,5 was fixed. The maximum time allowed for an animal to respond was 30 s. For the vocalization threshold (measured in mA), two stainless steel 30 gauge electrodes were inserted in the middle section of the tail. Electric stimulation was applied of 1 ms duration, containing 125 shocks of 1,6 ms width delivered from a high frequency square wave constant current generator. The maximal intensity of the current delivered was 2 mA.

Individual baseline tail-flick, hot plate latency and vocalization thresholds were determined in three pre-tests 20 min before drug administration.

Convulsive threshold

The onset of convulsive episodes in 2-3 s after pentylenetetrazole (50 mg/kg i.p.) and subsequent death in controls or animals pretreated with aqueous extract were evaluated.

Pentobarbital-induced sleeping time

The time elapsed between loss and recovery of the righting reflex was taken as sleeping parameter and recorded for control and drug-pretreated animals. Ten minutes after administration of extracts, mice were given an intraperitoneal dose of 50 mg/kg of pentobarbital.

Motility test

Spontaneous locomotor activity was recorded using an activity cage with automatic counting of the animals' movements across the bars on the cage floor. Naive mice were placed in activity cages for 30 min before injection and recording of activity. Motor activity was recorded for 120 min after treatment.

Pharmacological activity was tested by i.p. injection of the freeze-dried material dissolved in saline sterile solution in a volume not exceeding 0.2 ml.

Data concerning the yields of crude material and their activity are reported in Table 1.

TABLE 1

PLANT	Dry weight mg/g *	MDE mg/Kg	Activity ratio **
First ethanolic extract (IE)	58.1	463	1.0
Second ethanolic extract (IIE)	27.0	110	4.2
CELL CULTURES			
Callus extract	33.8	54	8.6
Suspended cell extract	20.4	84	5.5

* Expressed as mg extracted per g of fresh weight of leaves or cells.

** Activity of the extract compared with the first ethanolic extract of the plant (IE).

The first ethanolic extract of the plant (IE) or "fluid extract" may be considered a 50% ethanolic solution being obtained from fresh leaves, in which the content of water is 70-80% and an equal quantity (w/v) of 95% ethanol.

It appears as a green-dark solution that has a content in dry material corresponding to mg 58.1 per g of fresh leaves and is active in mice at the dose of 463 mg/kg.

The extracted leaves filtered from IE, when extracted again with 95% ethanol afforded an amount of solid material corresponding to 27.0 mg per g of fresh leaves. This extract (II E) administered i.p. in mice, shows an activity at the dose of 110 mg/kg four times higher than the previous extract IE. This means that in leaves an active lipophilic product is present that may be better extracted by 95% ethanol. With a single extraction with ethanol more than one-half of the activity produced by the P. incarnata plant is lost.

When callus cell cultures are extracted with 80% ethanol, the solid material gives 33.8 mg/g of fresh weight (see Table 1) corresponding to about one-half of that from leaves. This material, however, shows an activity corresponding to 54 mg/kg; over eight times higher than that of IE.

Cells from liquid cultures, similarly extracted, afforded 20.4 mg per g of f.w. with an activity at the dose of 84 mg/kg; 5.5 times higher than that of IE.

From the data reported above it is evident that, to isolate the structures produced by P. incarnata, the best source is callus or suspended cell cultures.

In a preliminary work in order to identify the active products, partitioning the crude extracts either from callus cultures or suspended cells cultures demonstrates identity in the behaviour of the active compounds present in the P. incarnata leaves. In fact, at least two different products are present also in cell cultures: one may be extracted with CH_2Cl_2 and one with n-BuOH, each with different behaviour on column chromatography and TLC.

None of these products can be ascribed to the suspected structures responsible for the activity, such as beta carboline (harmane derivatives), flavonoids or maltol.

Studies are in progress to identify the active ingredients present in cell cultures of P. incarnata with the aim of confirming their identity with those present in the plant and permit, in the future, their quantification in different pharmaceutical preparations as being responsible for the activity.

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