Journal of Advances in Science and Technology

Vol. IX, Issue No. XIX, May-2015, ISSN 2230-9659

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AN INTERNATIONALLY INDEXED PEER REVIEWED & REFEREED JOURNAL

www.ignited.in

Journal of Advances in Science and Technology
Vol. IX, Issue No. XIX, May-2015, ISSN 2230-9659

A Study on Extraction of Plant Material

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Abstract – All the three extracts of Calotropis procera (roots and aerial parts) showed promising cytotoxic effect in concentration dependent manner against a panel of thirteen human cancer cell lines of various tissue origins. The chloroform fraction of 95% alcoholic extract and n-butanol fraction of 50% aqueous-alcoholic extract from roots of Calotropis procera. The chloroform fraction of 95% alcoholic extract and chloroform & n-butanol fractions of 50% aqueous-alcoholic extract from aerial parts of Calotropis procera also exhibited promising cytotoxic effect in concentration dependent manner against a panel of twelve human cancer cell lines of various tissue origins. The active extracts and fractions of roots and aerial parts of Calotropis procera were more or less equally active.

The 50% aqueous-alcoholic and aqueous extracts of Cassia occidentalis (whole plant) exhibited more or less similar cytotoxicity in concentration dependent manner against panel of fourteen human cancer cell lines from various tissues. These extracts showed promising cytotoxcity only against few cell lines.

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EXTRACTION OF PLANT MATERIAL

Freshly collected plant material was chopped and shade dried. The dried coarse plant material was divided into three parts and extracted with 95% alcohol, aqueous- alcohol (1:1) and water. The respective extracts were named as 95% alcoholic extract, 50% aqueous- alcoholic extract and aqueous extract as shown in Fig 1. The method for the preparation of extracts is described below:

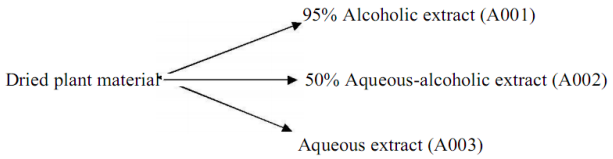

Fig 1 Scheme for preparation of extracts

1. Preparation of extracts by repeated solvent extraction procedure

Dried powdered coarse plant material was placed in a conical glass percolater, submerged with 95% alcohol at ambient temperature for 16 hours (overnight). The solvent was decanted, percolate collected and the process was repeated four times. Few drops of the last percolate were placed on a watch glass and allowed to evaporate, if no residue was left, the extraction was considered complete; otherwise it was repeated till complete extraction was obtained. The pooled solvent was concentrated on rotavapor under reduced pressure at 50°C. Final drying was done ini tially in a vacuum desiccator and finally in lyophilizer. The dried extract was scrapped off and after weighing transferred to a tared wide mouth glass container. Nitrogen was blown in the container before capping and stored at -20^{0}C under desiccation to yield alcoholic extract (A001). Similarly, 50% aqueous-alcoholic extract (A002) was prepared by soaking another lot of dried plant material in alcohol-water (1:1). The aqueous extract (A003) was prepared by heating the plant material with distilled water on steam bath for four hours, the extract was decanted and filtered through celite powder. The process was repeated four times using additional amount of distilled water, pooled extract was concentrated on rotavapour and dried in a lyophilizer. Nitrogen was blown in the container before capping and stored at -20^{0}C in deep freezer. The detail of various extracts prepared is shown in Table 2.

Table 2: Preparations of various extracts from selected plant material

Plant	Plant Part Used	Extract		
		95% Alcoholic	50% Aqueous-alcoholic	Aqueous
Calotropis procera	Root (P01)	P01/A001	P01/A002	P01/A003
	Aerial (P08)	P08/A001	P08/A002	P08/A003
Cassia occidentalis	Whole plant (P14)	P14/A001	P14/A002	P14/A003
Cuscuta reflexa	Whole plant (P14)	P14/A001	P14/A002	P14/A003
Eucalyptus citriodora	Leaves (P03)	P03/A001	P03/A002	P03/A003
Ficus hispida	Leaves (P03)	P03/A001	P03/A002	P03/A003

2. Sequential extraction procedure for *Eucalyptus citriodora*

In this method, freshly collected plant material (*Eucalyptus citriodora*) was chopped, shade dried and grounded. From the dried coarse plant material, six different extracts were prepared *viz.,* n-hexane, chloroform, ethyl acetate, ethanolic, 50% ethanolic and aqueous.

Table 3: Sequential extraction procedure for *Eucalyptus citriodora*

Plant	Part Used	Extract
Eucalyptus citriodora	Leaves (P03)	1110/P03/ n-Hexane
		1110/P03/ Chloroform
		1110/P03/ Ethyl acetate
		1110/P03/ Ethanol
		1110/P03/50% Ethanol
		1110/P03/ Aqueous

The method of preparation of extracts is as under:

2.1. Preparation of n-hexane extract

The dried plant material (500 gm) was taken in a stoppered conical flask (250 ml), vigorously shaken with n-hexane (100 ml) and allowed to stand for 30 min. The supernatant was decanted. Procedure was repeated thrice using fresh n-hexane every time. The combined n-hexane soluble portion was evaporated to dryness under reduced pressure at 50°C. The dried isolate was scrapped of f and transferred to an air tight glass container (Fig 2). Nitrogen was blown in the container before capping and the extract was stored at -20^{0}C in desiccator.

2.2. Preparation of chloroform extract

The residue left after removing the n-hexane soluble part was further macerated with chloroform (4 x 100 ml) as described for n-hexane extract. Combined chloroform soluble portion was evaporated to dryness under reduced pressure at 50°C. The dried isolate was scrapped off and transferred to an air tight glass container (Fig 2). Nitrogen was blown in the container before capping and the extract was stored at -20^{0}C in desiccator.

2.3. Preparation of ethyl acetate extract

The residue left after removing n-hexane and chloroform soluble part was suspended in water (200 ml). Suspension was taken in a separating funnel and extracted with ethyl acetate (4 x 100 ml). The combined ethyl acetate extract was evaporated to dryness under reduced pressure at 50°C. The dried isolate was scr apped off and transferred to an air tight glass container (Fig 2). Nitrogen was blown in the container before capping and the extract was stored at -20^{0}C in desiccator.

2.4. Preparation of ethanol extract

The residue left after removing n-hexane, chloroform and ethyl acetate soluble part was suspended in water (200 ml). Suspension was taken in a separating funnel and extracted with ethanol (4 x 100 ml). The combined ethanol extract was evaporated to dryness under reduced pressure at 50°C. The dried isolate was scr apped off and transferred to an air tight glass container (Fig 2). Nitrogen was blown in the container before capping and the extract was stored at -20^{0}C in desiccators.

2.5. Preparation of 50% ethanolic extract and aqueous extract

The residue left after removing n-hexane, chloroform, ethyl acetate and ethanol soluble part was suspended in water (200 ml). Suspension was taken in a separating funnel and extracted with 50% ethanol (4 x 100 ml). The combined 50% ethanolic extract was evaporated to dryness under reduced pressure at 50° C. The dried isolate was scrapped off and transferred to an air tight glass container (Fig 2). Nitrogen was blown in the container before capping and the extract was stored at -20^{0}C in desiccator. Finally aqueous extract was prepared by collecting filtered water fraction, centrifuged at 1000 rpm for 20 min and finally dried by freeze dryer.

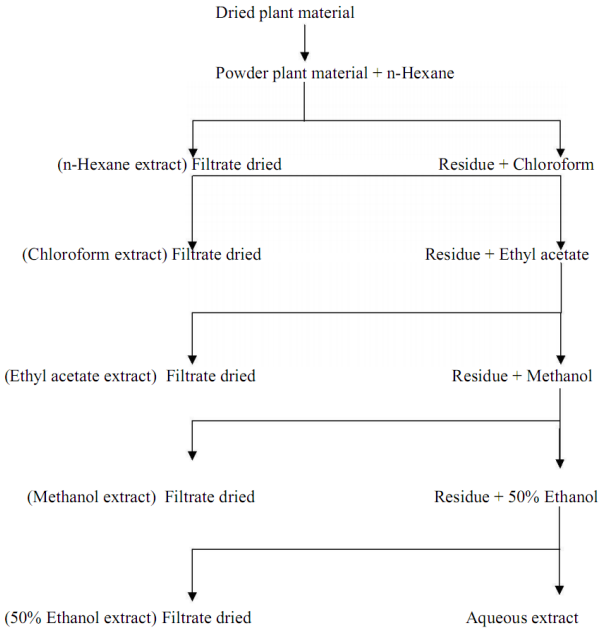

Fig 2 Scheme for extraction by sequential procedure

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