Extraction of two medicinally active compounds from plant sources

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Abstract

Asiaticoside (the ester glycoside of the triterpene *asiatic acid*) and madecassoside (the ester glycoside of the triterpene *madecassic acid*) are the chief medicinally active compounds of the plant *Centella asiatica* ("Mandukparni") of the family Apiaceae. These were successfully extracted through column chromatography along with a saponin fraction.

The alkaloid atropine, used extensively as a muscle relaxant, especially during eye surgery, was extracted from the common jimson weed – *Datura stramonium* of the family Solanaceae. On commercial scale, the compound is normally obtained from another plant of the family– *Atropa belladona*. Here, we report the extraction procedure for atropine from *Datura* as perfected by us.

Introduction

Centella asiatica (Linn.) Urban. is a common small weed (Fig. 1) found in moist places throughout India (Haines, 1925). According to Dey (1980), plant is known as "Brahmi" or "Mandukparni", and, its various parts are used in Ayurvedic preparations named "Brahmi-Pak", "Sarswat-arista", "Sarswat-ghrita",and "Brahmi-taila". The preparations are used as tonic, laxative, alterative, alexiteric, and antipyretic. Reports of their use in urinary discharges, leucoderma, anemia, bronchitis, inflammations, etc. are also available. The most outstanding claim regarding the plant is that the consistent use of its leaf powder improves memory (Kirtikar and Basu 1934). Triterpenes of the plant are known to be highly useful for skin care and toning, and, prevent pre-mature wrinkles (Kumar *et. al.*, 2007). Active chemical compounds of the plant are asiatic acid, asiaticoside, madecassic acid, madecassoside, fatty oil, sitosterol, tannin, a resinous substance, hydrocotylin (alkaloid), vellarine, pectic acid, ascorbic acid, two saponins – brahmoside and brahminoside, three triterpene acids – brahmic acid, isobrahmic acid and betulic acid and stigmasterol (Chopra *et al.* 1956; Rastogi *et al.*

1960). Schaneberg *et al.* (2003) extracted six triterpenes i.e. asiaticoside, madecassoside, asiatic acid, madecassic acid, terminolic acid and asiaticoside – B.

However, major active principles in *Centella asiatica* are the triterpenes - asiatic acid and madecassic acid, and, their derived triterpene ester glycosides, asiaticoside and madecassoside (Kartnig, 1988; Farnsworth and Bunyapraphatsara, 1992). Molecular formulae of asiaticoside and madecassoside are $C_{48}H_{78}O_{19}$ and $C_{48}H_{79}O_{19}$ respectively with M.P. between 230^0 – 233^0 C approx. Molecular weight of the compounds are 959.12 and 960.12 respectively. Structure of these compounds is presented in Fig.3.

Datura stramonium (Fig. 2) (Hindi name – "Dhatura", English name – Jimson weed/Thornapple) is a common shrub of the family Solanaceae, close to the belladona plant- *Atropa belladona*. The primary psychoactive substances in the plant are the alkaloids atropine and scopolamine (*http://www.a1b2c3.com/drugs/index.htm*). Atropine is a highly toxic alkaloid of tropane group. Apart from being used as a muscular relaxant and as an anti-spasmodic agent, atropine has been used in treating Parkinson's disease, peptic ulcers, diarrhea, bronchial asthma, and, also to treat nerve gas poisoning (Demeyer *et al.,* 1994). For treatment of patients following an organophosphate (OP) exposure after a terrorist attack using OP nerve agents, atropine is used recreationally (Bania *et al*., 2004). Structure of atropine is shown in Fig.4.

Extraction of asiaticoside and madecassoside from *Centella asiatica*

The compounds were isolated from other plant constituents by methanol extraction, and, separated from other terpenes by silica gel column chromatography. The protocol for the extraction was a modification of that followed by Matsuda *et al.* (2001).

Procedure:

(1) Air dry 500 gms. of plant leaves, (2) Powder the dried leaves with help from pestle and mortar, (3) Extract the contents with methanol, (4) Repeat step 3 two more times with the left-over residue, (5) Combine the three methanol extracts in a porcelain container, (6) Evaporate the solvent in an

oven under reduced pressure, (7) Weigh the dried extract, (8) Partition the extract in 1:1 mixture of ethyl acetate and water, (9) Dry the water dissolved fraction in an oven, (10) Extract the dried H_2O fraction with methyl alcohol, (11) Keep the methyl alcohol extract for silica gel chromatography, (12) Prepare silica gel column, (13) Prepare three elution mixtures of methyl alcohol and water in the ratio of 40:60, 50:50, and, 60:40, (14) Put the methyl alcohol plant extract into the silica gel column, (15) Obtain three fragments of compounds by the three elution mixtures in the given order, (16) Separate fraction 2 eluted by 50:50 elution mixture containing madecassoside approximately as 0.068%, (17) Separate fraction 3 eluted by 60:40 eluting mixture containing 0.99% asiaticoside, (18) Subject the two separated fragments to HPLC for obtaining pure madecassoside and asiaticoside.

Extraction of atropine from *Datura stramonium*

Atropine is racemic form of l-hyoscyamine. The latter can be racemised by heating with ethanol alkaline solution (Sinha *et. al.*, 2004).

Procedure of extraction of atropine

(1) Dry 500 gms. seeds of *Datura stramonium,* (2) Boil the seeds in water for 1-1.5 hours in a beaker, (3) Strain the extract through a fine pored mess, (4) Add 4 ml of conc. Sulphuric acid into the liquid, (5) Leave for half an hour, (6) Take out the clear layer of liquor with the help of a pipette, (7) Filter to obtain a transparent sherry-coloured solution, (8) Add ammonium carbonate to the solution till saturation, (9) Find colour change of the solution to black from sherry, (10) Leave to obtain crystals of atropine in 15-30 minutes, (11) Take out the upper supernatant with the help of a Pasteur pipette, (12) Dry the crystals on a filter paper, (13) Dissolve dried crystals into boiling alcohol, (14) Evaporate alcohol and obtain pure crystals of atropine.

Recommended uses of the extracted compounds

TTFCA / *TTF* (Total terpenoid fraction) containing about 36% asiaticoside is recommended to be used in for removing wrinkles, acne, mycosis, wound healing, venous insufficiency, sunburn,

blotchiness and skin toning. TTFCA is acutely effective in reducing capillary filtration (CF) and oedema in subjects with venous hypertensive microangiopathy (De Sanctis *et. al*., 1994).

Atropine has been established as a very effective remedial substance (antidote) to ward off the effects of nerve agents like tabun (GA), sarin (GB), soman (GD) and VX (Anonymus, 1990) . Nerve agents bind to part of the acetylcholinesterase (AChE) enzyme which controls the activity of acetylcholine – the neurotransmitter involved in muscular contraction. Atropine works by blocking one type of acetylcholine receptor so that the acetylcholine that is already in the synapse cannot work.

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Figures

Fig.1 *Centella asiatica*

Fig.2 *Datura stramonium*

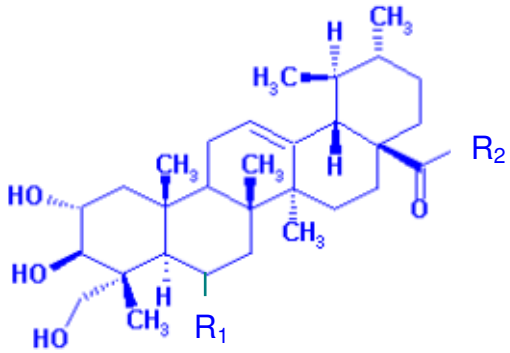

Fig.3 Structure of Asiatic acid and madecassic acid

Asiatic acid: R_1 = H and R_2 = OH

Madecassic acid: R_1 = OH and R_2 = OH

Trisaccharide *rha-glc-glc* moiety is ester linked to R_2 in glycosides

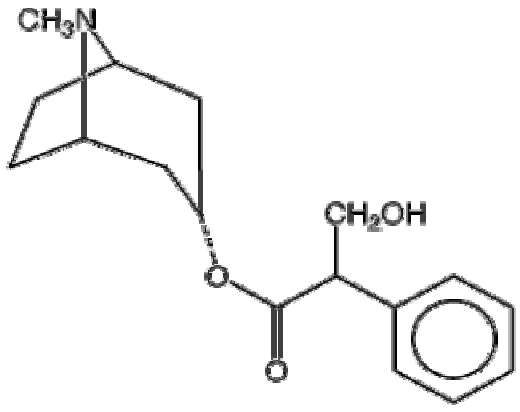

Fig.4 Structure of atropine