Chapter-5

SECONDARY METABOLITE PRODUCTION

Plants are the source of several natural drugs and hence these plants are termed as the medicinal plants. The medicinal plants rich in secondary plants products are termed as ***Medicinal*** or ***Officinal*** plants. These secondary metabolites or products exert in general, a profound physiological effect on the mammalian system and, thus are known as active principle of plants. A plant produces two types of metabolites.

1. Primary metabolites which get involved directly in plant growth and metabolism, viz. Carbohydrates, proteins, lipids etc. Primarily used as high-volume, low valve bulks chemicals, they are used as industrial raw material, foods or food additives for eg vegetable oils, fatty acids and carbohydrates etc.

2. Secondary metabolites are compounds biosynthetically derived from the primary metabolites but mere limited in occurrence in the plant kingdom, and may be restricted to a particular taxonomic group. Secondary metabolites are mostly accumulated by plant cells at particular developmental stages making their extraction and purification difficult (Ramavat 2000).

Plant cell culture was originally developed as a research tool in order to study the physiology and biochemistry of plants. However, as the technique developed a commercial potential was recognized and first to develop was micropropagation. Plant cell cultures were also found to produce compounds characteristic of the original plant. As plants contains a profusion of compounds which are used as dyes, pharmaceuticals, perfumes and insecticides, plant cell culture was proposed as an alternative supply of these compounds (Berlin 1988; Buitelaar & Tramper 1992; Verpoorte *et al* 1993; Jasrai & George 2000). Plants also contain a wide range of enzymes, and are thus of great interest for use in biotansfromations (Alfermann *et al* 1983). More recently plant cell cultures have been suggested as sources of enzymes (Kato *et al* 1991) and as expression systems (Hogue *et al* 1991).

Important alkaloid of plant origin and their pharmacological activity included Ajmalicine in *Catharathus roseus* as hypotensive, Berberine in *Coptis japonica* as antispasmodic and antiprotozoal, Colchicine from *Colchicum automnale* as antimitotic, Ephedrine from *Ephedra gerardiana* as spasmolytic, Morphine from *Papaver somniforum* as analgesic and sedative, Quinine from *Cinchora ledgeriana* as

antimalarial and Vinblastine in *Catharanthus roseus* as anticancer.

The members of umbelliferae or Apiaceae are important as spices or food adjuncts and are used for flavoring the day-to-day cooked food. These spices are aromatic due to the presence of essential volatile oil which give flavor to the food. These spices are also used extensively in Ayurvedic medicines. Some possess antiseptic properties and are used occasionally in cosmetics and perfumery (Chopra *et al* 1958). The seeds of *Trachyspermum ammi* (L) Sprague are used in diarrhoea, dyspepsia, colic and flatulence indigestion. It possesses carminative, stimulant, tonic and antispasmodic properties. The fruits have an aromatic smell and a pungent taste. The fruits yield colourless to brownish essential oil in which thymol is present to a large extent (36-60 %). Thymol- a phenyl propene type of phenolic compound, have an aromatic ring with attached three-carbon side chain. They are derived biosynthetically from the aromatic amino acid phenylalanine (Harborne 1984). Through-out India work on improvement of spices (Umbelliferous) has been scanty and disjointed (Hore, 1979). Thus plant cell and tissue cultures have been shown to be useful for not only studying primary, secondary metabolites and control mechanisms (Abou–Mandeur *et al* 1987) but also for improvement and enhanced production. The essential oil present in the seeds of *T. ammi* is used as insecticide and in indigenous medicines the annual production of over 35 metric ton is not sufficient enough to meet the present demands. Therefor, possible detection of thymol in cell cultures of *T. ammi* was investigated.

Method for extraction of Thymol

The different possible sources of thymol in cell culture i.e. fresh callus of various stages, dried callus, embryogenic callus, somatic embryogenesis derived plantlets, plants germinated from seeds of *T. ammi* and seeds were all exploited for detection of thymol.

The tissues were homogenized with glass powder in benzene and kept on shaker (100 rpm) for overnight. The debris were removed by centrifugation and the benzene extract was subjected to thin layer chromatography (TLC) with silica gel (250 u thick). A standard thymol was always run on each TLC plate. The silica gel plates were developed in benzene and Vanillin-H_2SO_4 spray was used to detect thymol, which appeared as magenta bands. The standard thymol band was obtained at a R_f value of

0.75. Similar bands with same R_f valves were obtained from plant material. An aliquote of 0.01 ml of each sample was used for separation and detection of thymol

Thymol was found to be present in various concentrations as seen by degree of colour development on TLC plates in different sources. Thymol was detected only at the stationary phase of embryogenic and nonembryogenic callus (Fig-12 a & b). This indicates an inverse relation between growth and secondary metabolite production accumulation (Barz *et al* 1990). Also it was found to be present in the regenerated plants (Fig-11 a). The separation of thymol was much better on 2-dimensional TLC (Fig. 11 d). The benzene extracts from non-embryogenic callus also showed presence of other unidentified compounds.

Quantification of Thymol:

The benzene extracts were also subjected to Gas Chromatography – Perkin Elmer 8700^0 with following specifications—

Column- 10 %, Carbowax- 20M.

Support on chromosorb WAW 80/100 mesh, S-S, 1/8" OD 3 Mtrs

Carrier gas Argon Carrier gas flow – 30 min.

Hydrogen gas flow – 300 ml/min.

Air flow – 300 ml/min.

Oven temperature – 185° C

Detector temperature – 230° C

Injector temperature – 230° C

Attn – 32,64

Sensitivity – high

Chartspeed – 3

Peak width – 4

Retention Time – Benzene 1.74 mint and Thymol 14.68 min

Injection volume – 0.2 ul

Fig-11 Separation of Thymol from in vitro cultures

a) Different concentrations of standard thymol

b) regenerated plants

c) embryogenic callus

d) 2-dimentional separation of thymol

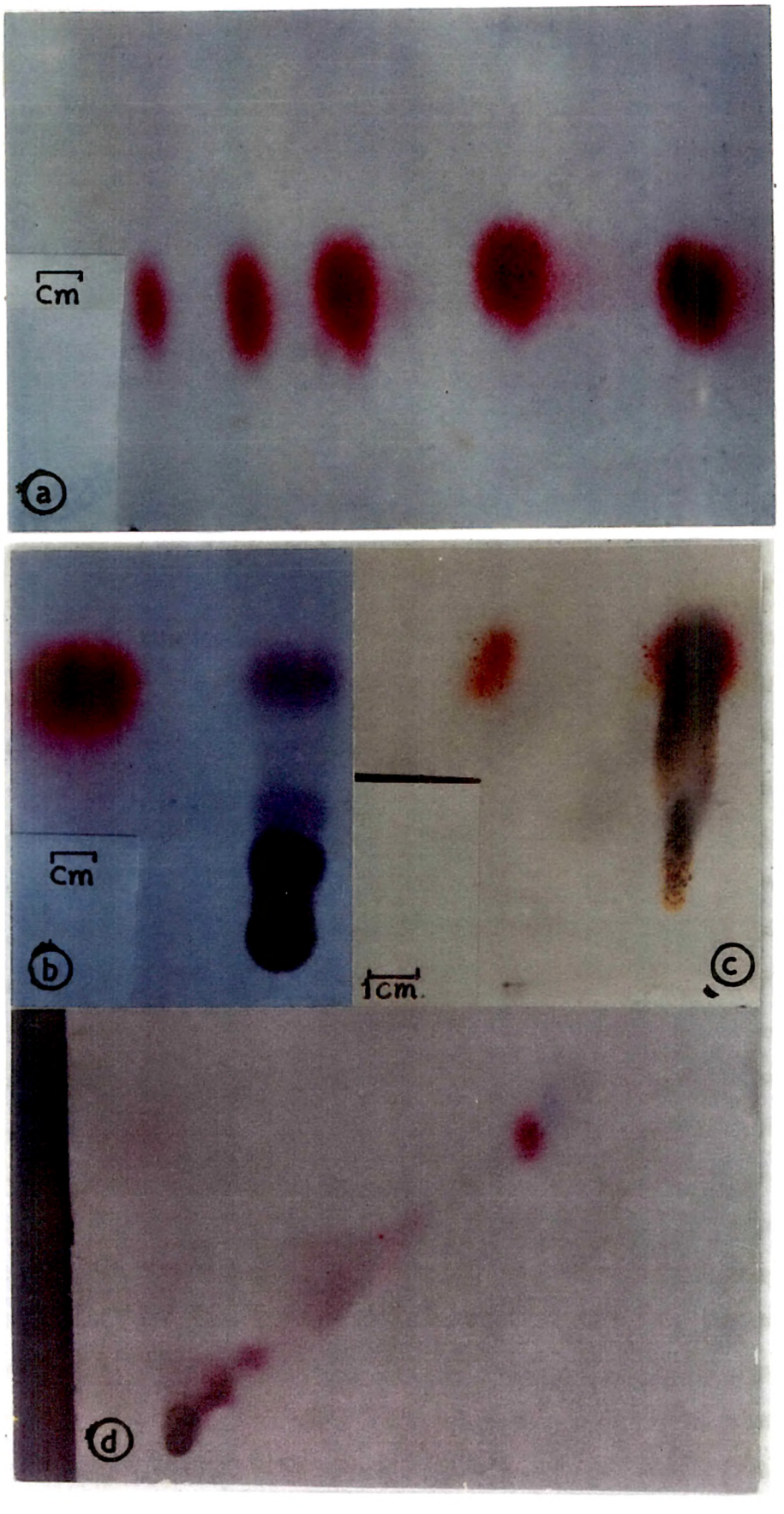
cm
a
cm
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Fig-12 Stationary phase of

a) embryogenic callus

b) nonembryogenic callus

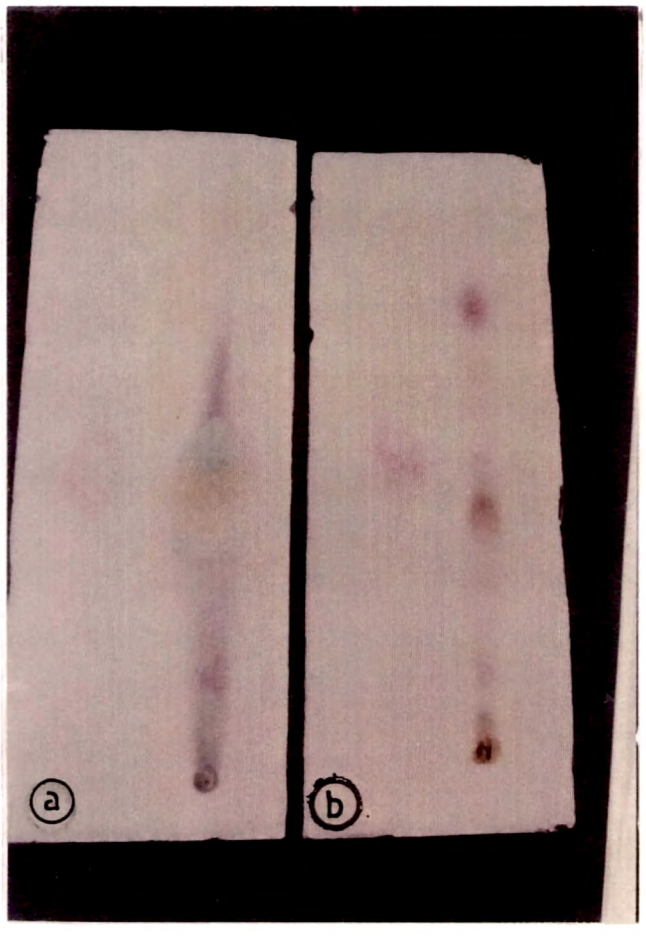
a
b

Table-5: Quantification of Thymol in different sources

Plant material	Thymol (%)
Fresh callus	0.04
Nonembryogenic callus	0.08
Embryogenic callus	0.2
Regenerated plants from embryos	1.6
In vitro raised seedlings	0.4
Seeds	3.07

The quantification of thymol in different plant sources showed almost 0.2 % of thymol in embryogenic callus compared to non embryogenic callus showing 0.08%. Similarly the regenerated plants raised from somatic embryos showed 1.6 % of thymol compared to the in vitro raised seedlings.

Since most of the thymol demands in our country are at present met by chemical synthesis, it would be rewarding to improve quantitatively and qualitatively the yield of thymol to meet the growing demand.