CHAPTER IV

A NEW AND RAPID COLORIMETRIC METHOD FOR QUANTITATIVE ESTIMATION OF DIOSGENIN IN THE YAM OF DIFFERENT SPECIES OF *Dioscorea.* AVAILABLE IN THE REGION

Introduction

Out of various steroidal precursus diosgenin has been noted to be the most important source of raw material for the synthesis of steroid drugs. (Applezwig, 1962; Bhatnagar and Puri, 1974). The discovery that diosgenin found in the yam of *Dioscorea* can be converted to progesterone (Marker et al 1947) has lead to the production of oral contraceptives on commercial scals. Since then different species of *Dioscorea* yielding diosgenin has so far been utilised for a considerable period of time.

The situation started changing after that period when rising prices and uncertainty of the availability of *Dioscorea* yams caused shortage of steroid drugs based on diosgenin (Aplezweig, 1`969; Love, 1976). For this reasons very recently much importance has been given on searching of alternative source of diosgenin and for which an easy and rapid method for quantitative estimation of diosgenin is required.

Methodology sofar used for the estimation of diosgenin are gravimetric (Selvaraj, 1971) gas liquid chromatography (Tang et al 1978, Glyzine et al 1981) densito metric method using TLC scanner (Gunawan et al 1994). Paseshnichenko et al (1978) utilised colorimetric method for the determination of glycoside of diosgenin. There is no information available in connection with the utilisation of a rapid colorimetric method for quantitative estimation of diosgenin from the yam of *Dioscorea* on microscale. Here an attempt has been made to work out a rapid colorimetric method for quantitative estimation of diosgenin taking minimum amount of plant tissue.

Materials and Methods

Material

Yams of *D.alata, D. kamoonensis, D.arachidna, D.sikkimensis, D. bulbifera, D.esculenta, D.sativum and D.prazeri.*

Method

Collection and preparation of yam of *Dioscorea*

Yams of different species of *Dioscorea* were collected and washed with water to free it from soil particles. They were cut into pieces, sundried and made to powder with the help of a grinder machine. The dry powdered samples were used for extractron of diosgenin.

Extraction of diosgenin from powdered yam of Dioscorea

The powdered sample of yam was refluxed with chloroform for 15-20 minutes and filtered. The filtrate contained free diosgenin. The chloroform part was concentrated. The residue of plant tissue obtained after filtration was dried to free it from chloroform and was subjected to acid hydrolysis with 11.3 (N) HCl for 5 hours and neutralised. The solid matter was dried and extracted with petroleum. ether. The extract was taken to dryness and the residue was dissolved in chloroform containing diosgenin which was initially present in bound form.

Purifiction of diosgenin by TLC

The chloroform part containing crude diosgenin was streaked on silica gel G and run in chloroform : acetone (3:1). The position of diosgenin on the plate was determined with the help of ;marker diosgenin. Silica gel powder was scraped off from the position of authentic diosgenin and was treated with glacial acetic acid it was slightly warmed and filtered. The filtrate was used for quantitative determination of diosgenin.

Determination of absorption maxima for reaction mixture

For determination of the absorption maxima, glacial acetic acid, Resorcinol (10,000 ppm) and conc. H_2SO_4 were used. Resorcinol solution was prepared after mixing 100 mg of resorcinol with 10 ml. of glacial acetic acid. 1 mg. of authentic diosgenin was taken in a test tube and dissolved in 1 ml. of glacial acetic acid. To this reaction mixture 1 ml. of resorcinol (10,000 ppm) and 0.2 ml. of conc. H_2SO_4 were added for the development of colouration to light pink. The solution of reaction mixture was allowed to stand for 15-20 minutes. With ;this coloured solution O.D. values at different wave length (n.m.) were determined with the help of spectro colorimeter (Systronics). The O.D. values of diosgenin at different wave length were plotted on a graph paper. Absorption maxima of ;diosgenin was determined.

Preparation of different grades of diosgenin

For the preparation of standard curve, the different grades of diosgenin solutions were prepared. 5 mg. of diosgenin was dissolved in 5 ml. of Glacial acetic acid to make 1,000 ppm. solution. From the stock solution a series of dilute solutions of 900, 800, 700, 600, 500, 400, 300, 200 and 100 ppm. were prepared after dilution with glacial acetic acid.

Preparation of stadnard curve

To each 1 ml. solution of diosgenin, 1 ml. Acetic resorcinol (10,000 ppm) and 2 ml. conc. H_2SO_4 were added for the development of pink colouration having an absorption maxima of 510 n.m. The reaction mixtures were kept at room temperature (27°C) for 15-20 minutes. Then the O.D. values of all solutions were determined with the help of colorimeter and standard curve was prepared.

Table 24 : Production of free and bound form of diosgenin in the yam of different species of *Dioscorea* in the ecological condition of Darjeeling and Sikkim Himalayas.

Species	Free forms of Diosgenin %	Bound form of diosgenin %	Total diasgenin %
D. alata	—	0.07	0.07
D. kamoonensis	0.20	0.28	0.48
D. arachidna	0.30	0.35	0.65
D. sikkimensis	0.25	0.35	0.60
D. bulbifora	0.06	0.74	0.80
D. esculenta	—	0.06	0.06
D. sativum	0.40	1.00	1.40
D. prazeri	0.60	1.60	2.2

Results and Discussion

Various ;methods applied so far in connection with the estimation of diosgenin are observed to involve gravimeteic determination (Selveraj, 1971) and Gas liquid chromatography (Glyzine, 1981). Though densitometric method using TLC seanner (Gunawan et al, 1994) has also been applied, but all these methods are cumbersome and sometimes involve costly machinary not available in all the Laboratories. Pasesnichenko et al (1978) utilised colorimetric method involving conc. H_2SO_4 and 1% formaldehyde to estimate glycoside of diosgenin and not as free diosgenin. Crude diosgenin after extraction is always associated with various other impurities. It has been observed that purification of diosgenin during estimation have not been taken into consideration in most of the cases. During isolation of diosgenin from bound form, acid hydrolysis of the tissue is the must. But during the treatment a considerable amount of diosgenin is lost due to conversion of diosgenin to its diene form which is generally considered waste in pharmaceutical industry (Harborne, 1973). Thus an attempt has been made to work out an easy and

rapid colorimetric method for quantitative estimation of diosgenin after being purified following the method of chromatography.

The procedure which has been worked out for quantitative determination of diosgenin is based on the principle of Bell and Briggs (1942) who noted that some steroidal compounds when treated with resorcinol in acetic acid followed by H_2SO_4 treatment produced characteristic colour. He utilised chemical test in connection with the detection of cholesterol. The proposed method is supposed to be a new and easy one in comparison to those mentioned earlier. More over in this method purification of diosgenin by TLC has been stressed. The colour complex was determined to have absorption maxima at 510 nm (fig.40) and the colour was stable after standing the mixture for 20 minutes and continued to last for 40 minutes. Diosgenin was calculated from the prepared standard curve ranging from 100 ppm to 1000 ppm of solution which was observed to obey Beer's law (Fig.41) the propsed method is supposed to be advantageous because of the fact that diosgenin can be determined from a low concentration of 100 ppm solution. Moreover with the help of this methods only a few milligramme of dried plant material was observed to be sufficient for estimation of diosgenin and it is observed to take a very small duration of time for the estimation of diosgenin dealing with large number of samples.

Most of the authors estimated diosgenin being isolated after acid hydrolosis of the yam of *Dioscorea*. As a result the diosgenin content reported earlier represented the total of free and bound form of diosgenin in the material studied. The proposed method has the advantage to estimate free and bound form of diosgenin and due to involvement of purification of diosgnin by TLC, it is expected to give more accurate result as compared to most of the methods involving estimation gravimetrically. The toble 23 shows the percentage yield of free as well as of bound form of diasgenin in the yam of different species of *Diascoreas* collected from natural habitat condition of Darjeeling and Sikkim Himalayas. It is very intwistifognot that the *D. alala* and *D. esculenta* which are general utilised by the local people as food does not contain the free diasgenin as compared to others i.e. *D. kamoonenis*

(0.20%), *D. arachidna* (0.30%) *D. sikkimensis* (0.25), *D. bulbifera* (0.06%) *D. sativum* (0.40%) and *D. prazeri* (0.60%). Otherwise the total value for some of the species of *Dioscorea* (Table 23) of diosgenin are slightly less than those observed (Table 14) after following the method of Bammi and Randhawa (1975). This is probably due to purification of diosgenin by TLC.

SUMMARY

A new and rapid colorimetric method has been worked out on the basis of development of colour when diosgenin is treated with resorcinol in acetic acid followed by conc. H_2SO_4.

Free form of diosgenin has been isolated when dried powder of yam of *Dioscorea* sp. has been refluxed in presence of chloroform for 15-20 mins. Bound form of diosgenin is obtained when powdered yam is subjected to acid hydrolysis, neutralised and extracted with pet. ether to obtain bound form of diosgenin.

The reaction mixture, consists of 1 ml. of diosgenin dissolved in glacial acetic acid, 1 ml. of resorcinol (10,000 ppm) and 0.2 ml. of conc. H_2SO_4. The solution of reaction mixture is allowed to stand for 15-20 min.

The absorption maxima has been noted to be 510 nm. The standard curve from 100 to 1,000 ppm. of diosgenin obey the Beer'slaw.

Stability of colour of reaction mixture remaines stable for forty mins. at room temperature (27^0C).

In connection with purification of diosgenin TLC has been applied in chloroform and acetone mixture (3:1 v/v).

The proposed method may be considered as an easy and rapid one and with the help of it, the free and bound form of diosgenin has been estimated in each of the yam of eight species.

Only *D.alata* and *D.esculenta* have been noted not to contain any free form of diosgenin but maximum amount of bound form of diosgenin on dry weight basis have been observed in this yam of *D.sativum* (1.00%) and *D. prazeri* (1.60%) growing in Darjeeling and Sikkim Himalayas.

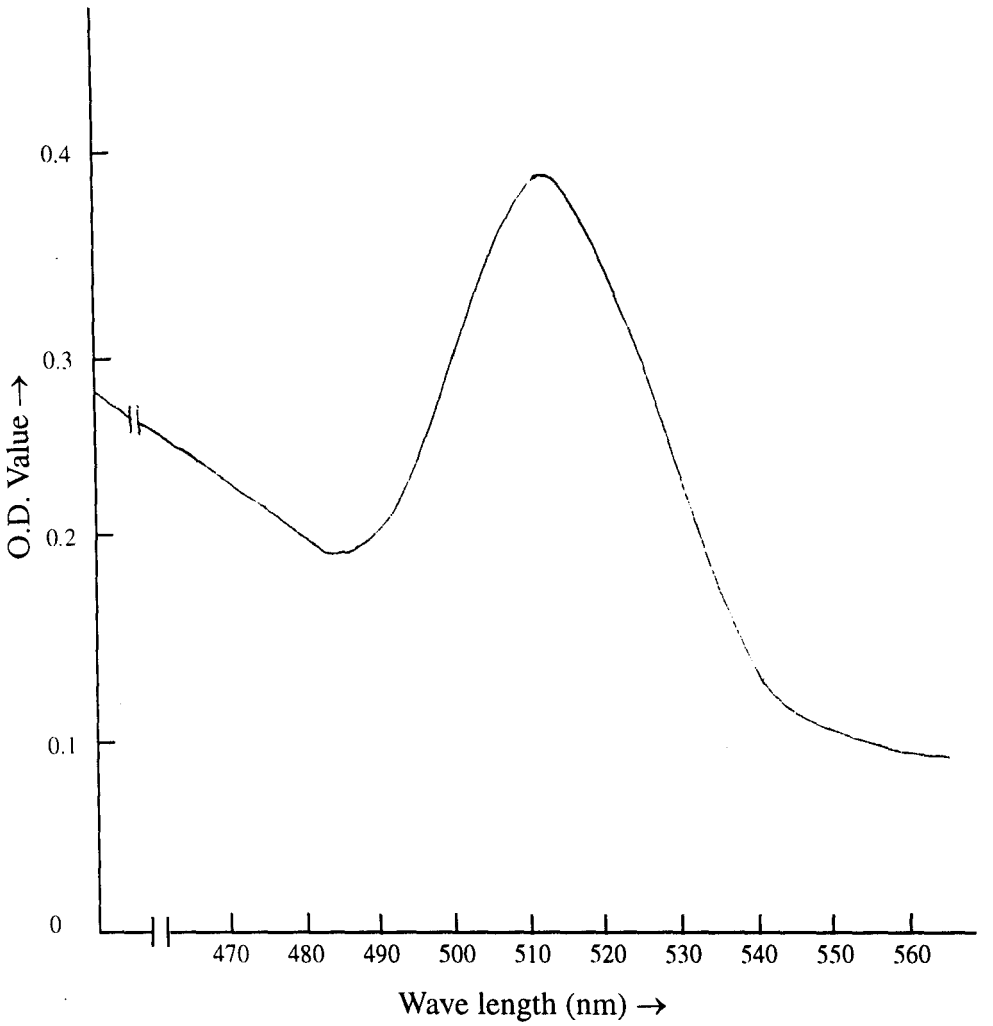

Fig. 40 : Absorption Maxima of Diosgenin (λ max 510 nm)

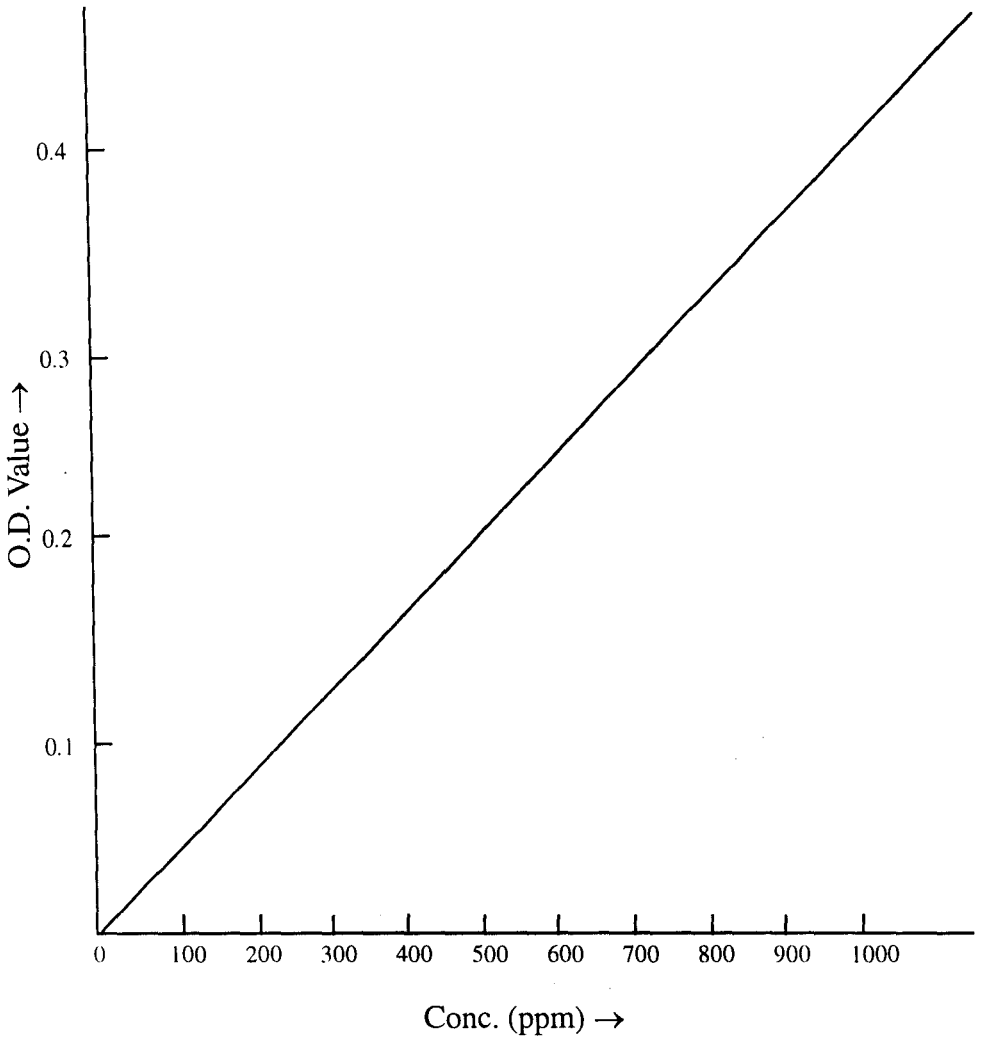

Fig. 41 : Standard curve of Diosgenin