CHAPTER — 5

Presence of Saponin in Mahuda flowers, invitro pharmacological investigations and nutritional study on steam treated Mahuda flowers

Experiment V

Objective 5(a) To isolate and identify saponin present in Mahuda flowers.

Objective 5(b) To conduct pharmacological investigations to find out invitro effects of alcoholic and water extracts of Mahuda flowers on isolated duodenum of rabbit and fundus of rat.

Objective 5(c) To explore the possibility of removing saponin from Mahuda flowers by steam treatment and evaluating the nutritive quality of steam treated flowers in terms of growth of weanling rats.

Highlights of the results

The Mahuda flowers contain 2.56% crude isolate saponin. The Rf value of Mahuda flower saponin was found to be between 0.56 to 0.67. The alcoholic and water extracts of Mahuda flowers containing varied concentration of saponin generated irritation on the smooth muscles of the rabbit duodenum and rat fundus resulting in a moderate non-reactivity of the tissues and inhibiting the normal pendular movement. The process of steaming followed by oven drying could not detoxify Mahuda flower saponin and/or an other toxicant present therein.

Introduction

Mahuda seed, locally known as Doli yields about 40 to 50% oil. Mahuda seed cake is considered unsuitable for incorporation in cattle and poultry feeds because it contains 7% saponin (Mulky and Gandhi 1977). It has also been reported that the saponin in Mowrah seed meal are much toxic than the tannins in sal meal (Menon 1977). Menon had demonstrated that rats fed diet containing 10% Mowrah meal died within a month due to the combined effects of starvation and toxicity. The author had further stated that Mowrah saponin are so toxic that they have been used to destroy fish in ponds and worms on lawns. Much earlier, Heywood and Ion (1940) had also reported that saponin present in the Mowrah seed meal are highly toxic.

Saponins are glycosides with a sugar and an aglycone moiety joined together. The group attached to the sugar in a glycoside is often referred to as the aglucone or aglycone (Mulky 1976). The aglycone of Mowrah saponin (sometimes referred to as Mowrin) has been termed as bassic acid (Liener 1975). Many glycosides occur in roots, barks, fruits and leaves of various plants (Moore et al 1911). Glycosides are usually well crystallized, colourless bitter solids, soluble in water and alcohol (West et al 1966). It has been reported that the groups attached to the sugars in the natural glycosides are generally, quite complex. However, the union is always through condensation of an alcoholic or phenolic hydroxyl with the glycosidic hydroxyl of the sugar.

Many authors have elucidated the triterpenoid structure of saponin (Heywood and Ion 1940, Harihoran et al 1972, Harborne 1973, Mulky 1976, Mulk, and Gandhi 1977). The triterpenoids are the compounds with a carbon skeleton based on six isoprene units which are derived

biosynthetically, from the acyclic C30 hydrocarbon, squalene (Harborne 1973). The triterpenoids are stated to be colourless, crystalline substances. They often have high melting point, and are optically active. But they are generally difficult to characterize because of their lack of chemical reactivity. Saponins are glycosides of both triterpenes and sterols and have been detected in over seventy families of plants (Baas and Raslogi 1967). The authors reported that glycosidic patterns of the saponins are often complex, many have as many as five sugar units attached, glucuronic acid being the common component. The two major classes of saponins, according to structural formulas, are the triterpenoids found in sugar beets and the steroid saponins represented by diosgin (George 1965). The presence of saponins in spinach, asparagus and horse chestnut has also been demonstrated by George (1955).

The saponins have been classified on the basis of their activity & structure. Alfalfa saponins are comprised of atleast three different types (Pederson et al 1967), and soybean saponins have been seperated into five fractions which differ in their activity (Birk 1964). In 1965, George had demonstrated that saponins have a property to inhibit trypsin and proteineses thereby, they could limit the digestibility and utilization of proteins (Goodhart and Shils 1980).

According to Walter et al (1955) saponins are bitter in taste, exhibit piscicidal action and are surface active agents with soap like properties and can be detected by their ability to cause foaming and haemolysis of blood. The haemolytic index (HI) of the chromatographically purified Mowrah seed saponin was assessed by Mulky in 1976. The author suspended 1 ml of rabbit red blood cell in

1 ml of isotonic buffer containing various concentration of 0.1 to 0.8 mg saponin. One millilitre of buffer plus 1 ml of cell suspension served as the control. The total weight of the reaction mixture (Vic 2g) divided by the smallest weight of saponin which caused complete breakdown of all cells (full haemolysis) was expressed as the haemolytic index. The Mowrah saponin in amounts greater than 0.4 mg caused full haemolysis at the end of 10h. Thus the haemolytic index of Mowrah saponin was calculated to be 5000.

Industrially, saponins are used as foaming agents in root beer and other frothy drinks (Mailer and Lopez 1947). George (1965) had demonstrated the use of saponin in commercial synthesis of steroidal hormones. According to Basu and Rastogi (1967) the search for saponins in plants had been stimulated by the need for readily accessible sources of sapogenins which could be converted in the laboratory, to animal sterols of therapeutic importance. Preston (1964) had earlier opined that the saponins present in several species of Dioscorea, are major source of starting material for the commercial synthesis of progesterone and other steroid products. Saponins have the capacity to form stable complexes with cholestrol and other 3-beta hydroxy -steroids, thereby, exhibit cholesterol lowering property. (Sirtori et al 1977).

Mully and Gandhi (1977) conducted acute toxicity studies in mice by administering orally and parenterally lethal dose (LD50, 50 mg/kg bod. weight) of Mowrah seed saponin. The results indicated that Mowrah saponin was extremely toxic when administered parenterally. The authors explained that acute toxicity produced by the parenteral route could be due to massive haemolysis caused by the saponin resulting in death of the animals due to anoxia. However, in humans

there is no direct evidence that saponins can be harmful when ingested as a minor component in the normal diet.

Recently, Joshi et al (1984) examined the acceptability and feeding potential of unprocessed Mahuda seed cake (UMSC) in the ration of buffaloes. The buffaloes fed for 60 days, feed mixture containing UMSC beyond 50% level exhibited depressed appetite. The authors observed that the digestibility of dry matter, crude protein and crude fibre declined significantly when the feed mixture contained UMSC at 75% level. They attributed depression in the digestibility of nutrients to the presence of 4.5% crude saponins and 5.0% tannins in the Mahua seed cake.

Saponin present in alfalfa (lucerne) to the extent of 2-3%, were held responsible for depressant effects on feed consumption, on growth and on utilization of diets in chicks (Cooney et al 1948 and Leplovsky et al 1950). Dried alfalfa meal when included in the diet at levels as low as 10%, was reported to cause retardation of growth in young chicks as well as depression of egg production in layers (Heywang 1950). As a matter of fact, Heywang and Bird (1954) have reported that incorporation of alfalfa saponin at graded levels in the diet showed that the level as low as 0.3% caused inhibition of growth in chicks. Later Heywang et al (1959) reported that in laying hens 0.4% saponin extracted from alfalfa or 2.2% saponin supplied as dried alfalfa meal depressed diet consumption followed by depressed egg production. It has been observed that when saponin was withdrawn from the diet, egg production was gradually restored to normal.

Many studies have been conducted to observe the toxic effects of Mowrah seed saponin in rats. Pradhan et al (1976) demonstrated that

a single dose of concentrated saponin extract of Mowrah meal administered through stomach tube in rats, produced acute intense inflammation of the intestine with sloughing of the superficial epithelial cells within a few hours after dosing. Mull. and Gandhi (1977) demonstrated that Mowrah saponin was extremely toxic when administered intraperitoneally. The authors explained that when saponin was orally ingested, it perhaps was not absorbed directly, but caused destruction and sloughing of the superficial layers of the intestinal mucosal membrane followed by intense inflammation thereby resulting in some degree of absorption of saponin through damaged hyperaemic tissues. Earlier Lindahl et al (1954) and Anderson (1957) had demonstrated that in animals, most saponins were poorly absorbed through the intestines. They reported that local effects produced by saponins caused death of the tissue due to inflammation of the alimentary canal. The intense surfactant activity of Mowrah saponin which is intensely irritant to mucous membranes has been demonstrated by Menon (1977). The author stated that the irritant effects on the nasal mucosa manifested by repeated bouts of sneezing resulted when an operator sprinkled the lawns with Mowrah cake as insecticide. Also, in our previous work a growth retardation was observed in weanling rats fed for 28 days, diets containing pressure cooked Mahuda flowers providing 18 or 36 g of carbohydrates (Pejaol et al 1984).

The pharmacological effects of Mahuda seed saponins, on isolated rabbit duodenum and rat stomach fundus have indicated that the addition of saponin into organ bath caused stimulant (irritant) activity, followed by death of the tissue (Mully and Gandhi 1977). This irritant effect was found to be related to the concentration of saponin in the bath. The saponin produced no effect on isolated

rabbit duodenum mounted in Dale's organ bath up to 1 mg in 20 ml bath fluid. At 2 mg, also there was no stimulant response but the normal rhythmic movements of the tissue were inhibited. At 5 mg, the saponin produced a slow contraction of the duodenum and the normal pendular movement was almost completely inhibited. With subsequent additions of saponin or acetylcholine which is known to cause muscle contraction, the tissue remained either non-responsive or gave a much diminished response. However, saponin produced no effect on the isolated fundus strip from rat stomach, upto a concentration of 5 mg in 20 ml bath fluid but at 50 mg, it produced a strong contraction.

Since, Mahuda seed cake contain 7% saponin, it was hypothesised that the Mahuda flowers being part of the same tree, might also be containing some amount of saponin which could have been responsible for depressed growth rate and lower food intake observed in the weanling rats, lower maternal weight gain in the pregnant rats and depressed growth in pups of the lactating rats (Chapter 3 & 4). Also, stimulant effect of Mahuda seed saponin had been observed on rabbit duodenum and rat fundus. The steam treatment has been used to destroy saponins present in plants. Thus an attempt was made

(a) to isolate and identify saponin if present, in Mahuda flowers (objective 5-a)

(b) to conduct pharmacological investigations to explore invitro, the effects of alcoholic and water extracts of Mahuda flowers on isolated duodenum of rabbit and stomach fundus of rat (objective 5-b).

(c) to explore the possibility of removing saponin from Mahuda flowers by steam treatment and evaluating the nutritive quality of steam treated flowers interms of growth of weanling rats (objective 5-c).

Materials and Methods

The experiment was conducted in three separate studies. Study 1 was designed to isolate and identify saponin if present in Mahuda flowers. Study 2 determined, invitro, the effects of alcoholic and water extracts of Mahuda flowers on the rabbit duodenum and on the rat stomach fundus. In study 3, investigation was carried out to evaluate nutritional quality of steam treated Mahuda flowers in terms of growth of weanling rats.

Analytical procedures

1. Identification of Mahuda flowers saponin

The method for isolating saponin from Mahuda flowers was that described by Mulay and Gandhi (1977). Fifty millilitre of distilled water was added to 5g of sundried Mahuda flowers and the mixture was allowed to stand for 24 hours. One hundred millilitre of 95% ethyl alcohol was then added and the mixture was put on a shaker for 24 hours. This was followed by the addition of 30 ml of 95% ethanol and the total volume of the liquid was made to 250 ml with water. the final strength of alcohol was 50%. The liquid was allowed to stand 24 hours and was filtered. Two gram of activated charcoal was added to 100 ml of the alcoholic extract which was warmed over steam for 15 minutes with occasional stirring. It was filtered and the residue washed with 200 ml of 50% ethanol. To the filtrate, 5 g of activated charcoal was added and the mixture was stirred and warmed over steam for 5 minutes. Again it was filtered and the charcoal washed successively, with 10 ml of 10 and 20% ethanol. The filtrate and washings were discarded and the absorbed

saponins were eluted from the charcoal, using a column containing a mixture of pyridine and absolute ethanol (3 : 7, v/v). The charcoal in the column was not allowed to dry between two successive additions of the solvent. The first portion of the elute (approximately 10 ml) was tested with Liebermann-Burchard reagent for the presence of saponin. The first elute was used to identify and separate saponin by, thin layer chromatograph, techniques.

2. **Thin layer chromatography technique (TLC)**

The thin layer chromatographical method used was that described by Stahl (1969). A sample spot was made near one end of the TLC plate (For preparation of TLC plates see Appendix II) and it was allowed to dry. The plate was then placed with this end dipped in the solvent mixture, taking care that the sample spot was not immersed in the developing solvent which was a mixture of chloroform-Methanol-Water (65 : 35 : 10). As the solvent moved towards the other end of the plate, the sample spot separated into various components. The plate was removed after an optimal development time for the region of 2/3rd height of the plate, and was then allowed to dry. The spots/zones were detected using a mixture of locating reagent (spraying solution) of sulphuric acid and acetic anhydride reagent (95% acetic anhydride and 5% sulphuric acid). The identification of the Rf value was based on following equation

$$Rf = \frac{\text{distance moved by the substance from origin}}{\text{distance moved by the solvent front from the origin}}$$

7. Quantitative isolation of Mahuda flowers saponin

Five gram of dried Mahuda flowers were refluxed with 100 ml of 2N HCL for 2 hours (Staud 1969). The mixture was allowed to cool and was filtered. The residue was neutralized by passing dilute ammonia through the filteration flask. The filter paper having the residue was allowed to dry in an oven at 60° C for 1 hour. The saponins were extracted from the residue on the filter paper, with petroleum ether in soxhlet apparatus for 24 hours. The solvent was allowed to evaporate in preweighed beaker on boiling water bath. The beaker containing the residue and the amount of isolate saponin was arrived at by subscracting the beaker weight.

Pharmacological studies (study 2)

The rabbit and rat weighing between 1.5 kg and 200 g respectively, were used for the experiments. The rabbit duodenum and rat stomach fundus were isolated and were washed with the perfusion fluid. The specimens about 4 to 5 cm in length were mounted in physiological salt solution (PSS'Locke, for preparation see Appendix III) using isolated organ bath as described by Ghosh (1971).

The effect of adding different concentration of saponin present in alcoholic and water extracts of Mahuda flower was investigated on rabbit duodenum and rat stomach fundus. These effects were recorded on the slow moving drum using the isotonic frontal lever and were compared against those of Acetylcholine (10 mcg/ml) which has a characteristic contractile property to produce muscular contraction.

Alcoholic extraction of Mahuda flowers was obtained by soaking 10g of dried Mahuda flowers in 100 ml of ethanol (95%). The supernatent

was filtered after 24 hours. One millilitre alcoholic filterate of Mahuda flowers equalled 100 mg of Mahuda flowers.

For water extraction, 25 g of Mahuda flowers were soaked in 75 ml of distilled water for 48 hours. The supernatent was filtered and made to 100 ml with water. One millilitre of water extract of Mahuda flowers equalled 250 mg of Mahuda flower.

Nutritional quality of steam treated Mahuda flowers (study 3).

Six weanling albino male rats of the Wistar strain weighing between 30 to 40 g were fed for 28 days, diet containing 25 g of Mahuda flowers powder (to provide 18 g of the carbohydrate), made from Mahuda flowers which were steamed for 30 minutes and dried in oven at 80° C for 3 to 4 days (25M30 diet/group). The composition of the 25M30 diet remained same as that of 25M20 diet described in Chapter 3, Table 3.1. The data obtained on growth rate, food intake and weight of the organs, was compared with the corresponding values of weanling rats fed bengalgram (BG diet) or 20 minutes pressure cooked Mahuda flowers (25% level) diet (25M20 diet) (Chapter 3).

Preparation of the Mahuda powder : Cleaned 100 g sun dried Mahuda flowers were steamed for 30 minutes in a colander (a container having holes) inserted in a big vessel which was 1/4th filled with water. The soft steamed flowers were then spread on a clean filter paper and fan dried for 1 hour. The partially dried flowers were then cut into small pieces to promote quick drying in an oven. The small pieces were then spread evenly on a tin foil and oven dried at 80° C for 3-4 days. The low moisture flowers then were kept in the desiccator for 1/2 hr which helped to cool the flowers and absorb the surface moisture. The crisp dry flowers so obtained were ground

into a fine powder and the powder was stored in air tight plastic bottle. The 100 g sun dried Mahuda flowers equalled 80 g of Mahuda powder. The Mahuda powder was incorporated in the 25M50 diet at the level of 25 g per 100 g diet.

Results and Discussion

The experiment V was conducted in three seperate studies. Study 1 was conducted to isolate and identify saponin if present, in the Mahuda flowers, using column and thin layer chromatography techniques. In study 2, pharmacological investigations were conducted to explore invitro, the effects of alcoholic and water extracts of Mahuda flowers on isolated duodenum of rabbit and fundus of rat. Stud. 3 was carried out to evaluate the nutritional quality of the steam treated Mahuda flowers.

The first elute treated with charcoal, collected through column chromatogram (study 1) gave positive reaction with Liebermann Burchard reagent (appearance of pink colour) confirming the presence of saponin in the Mahuda flowers.

The TLC technique applied to separate saponin exhibited the presence of saponin on the plate which was identified by the appearance of pink coloured band with brown dot after spraying the location reagent. The pink coloured band started disappearing after 5 minutes at room temperature but on heating the plate for 20 minutes in an oven at 70° C temperature, the colour started to reappear. The photograph of TLC plate (Figure 5.1) indicates the origin, the solvent front and the position of pink bands with brown dot. This photograph of the plate was taken using tracing paper with markings at the origin, solvent front, bands and dots with colours observed

Figure 5.1 Photograph of TLC plate showing the presence of saponin in Mahuda flowers.

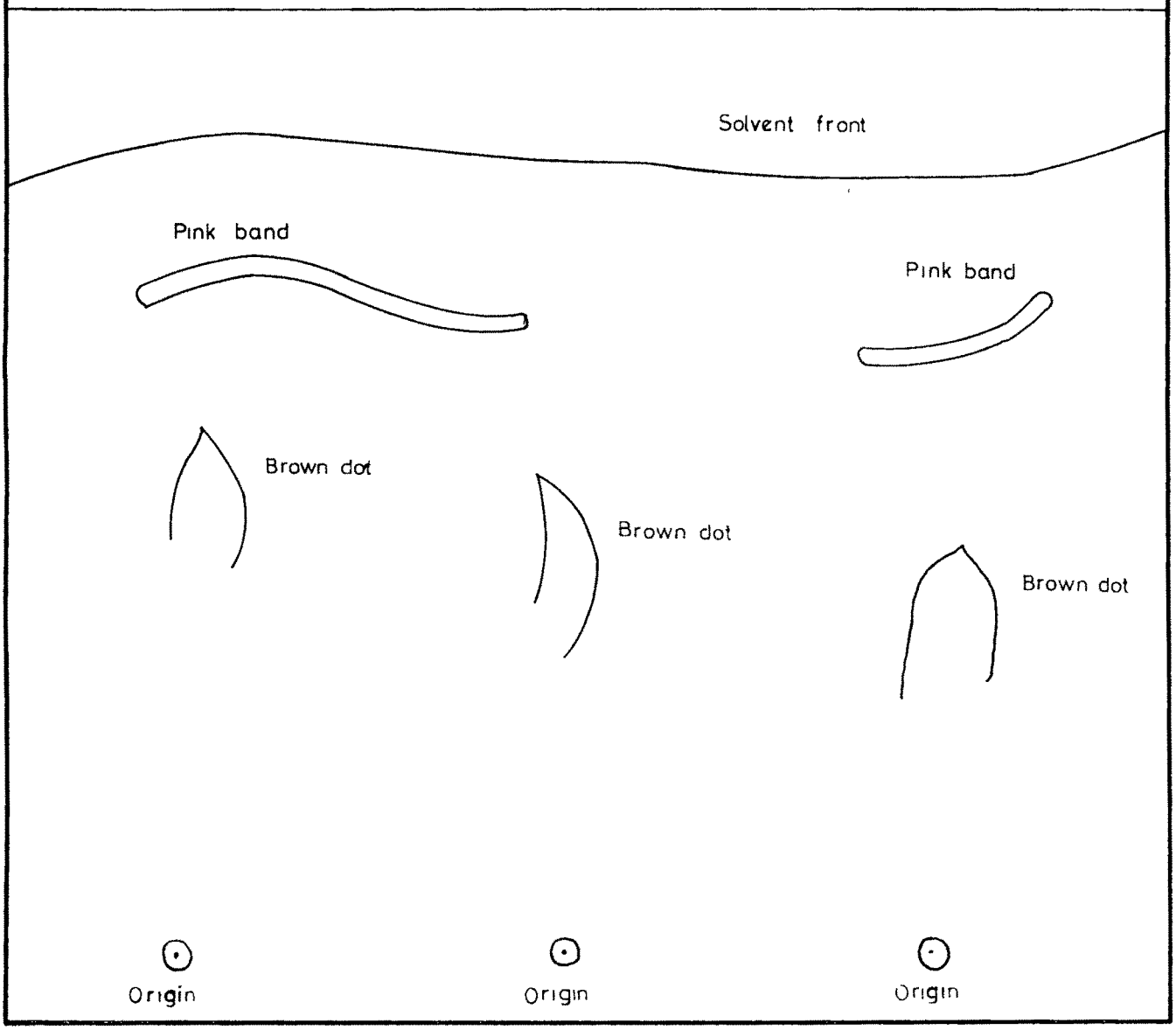
on the TLC plate at the time of experiment to capture the exact position of saponin band recovered on the TLC plate.

Figure-5.2 shows that the saponin band was positioned in the region from about Rf 0.56 to 0.65. Earlier, Mulay and Gandhi (1977) used n-butyl alcohol : M ammonium hydroxide : 95% ethanol (60 : 30.5 : 15) as the developing solution and found that the position of Mowrah seed saponin was within the region of Rf 0.15 to 0.50. Harborne (1973) had used a mixture of chloroform : Methanol in the ratio of 4 : 1 as the developing solvent to determine Rf values for various sapogenins. The author reported that the Rf value of Diosgenin was 0.55, of Tigogenin was 0.56, of Smilagenin was 0.62 and of Sitogenin was 0.16. The author also observed that the Rf values differed according to the polarity of saponins in various developing solvents such as Chloroform : ethanol (1 : 1) or hexane : methanol (4 : 1) or acetone-hexane (4 : 1) or chloroform : carbon tetra chloride : acetone (2 : 2 . 1). Kawasaki and Miyahara (1953) had earlier, opined that saponins are much more polar than the sapogenins because of their glycosidic attachments and are more easily separated by paper chromatography or thin layer chromatographic techniques. The authors opined that the TLC using silica gel proved to be successful technique in solvents such as butanol saturated with water or chloroform : methanol : water (17 : 7 : 2). Menon (1977) reported that acid hydrolysis destroyed the surfactant and haemolytic activity of Mowrah saponin by converting the saponin into sapogenin, with the activity of enzyme sapogenase glycosidase present in Mowrah meal. Mulay and Gandhi (1977) has shown that the slower-moving saponin (Rf 0.6) was converted by the acid hydrolysis process into the faster-moving saponin (Rf 0.94).

Figure:5-2 Tracing of TLC plate showing position of saponin separated from mahuda flowers.

Solvent system- Chloroform : Methanol : Water
(65:35:10)

Spraying reagent-Acetic anhydride : Hydrochloric acid
(95:5)



In the present study the Rf value of 0.56 of Mahuda flower saponin corresponded to that of Tigogenin and Smilagenin variety of sapogenins. However, the Rf value of 0.56 was not very far from that of 0.55 of Mahuda seed saponin reported by Mully and Gandhi (1977). In addition, Mull. and Gandhi (1977) have identified and characterised Mowrah seed saponin as triterpenoid compounds with a carbon skeleton based on six isoprene units. Earlier, Van Atta (1967) had obtained the Rf value of 0.55 for the alfalfa saponins and had characterised some of the alfalfa saponin components as triterpenoid compounds. The Mahuda flower saponin observed in the present study may, therefore, be triterpenoid in configuration.

Quantitative isolation of Mahuda flowers

Saponin and foam forming test

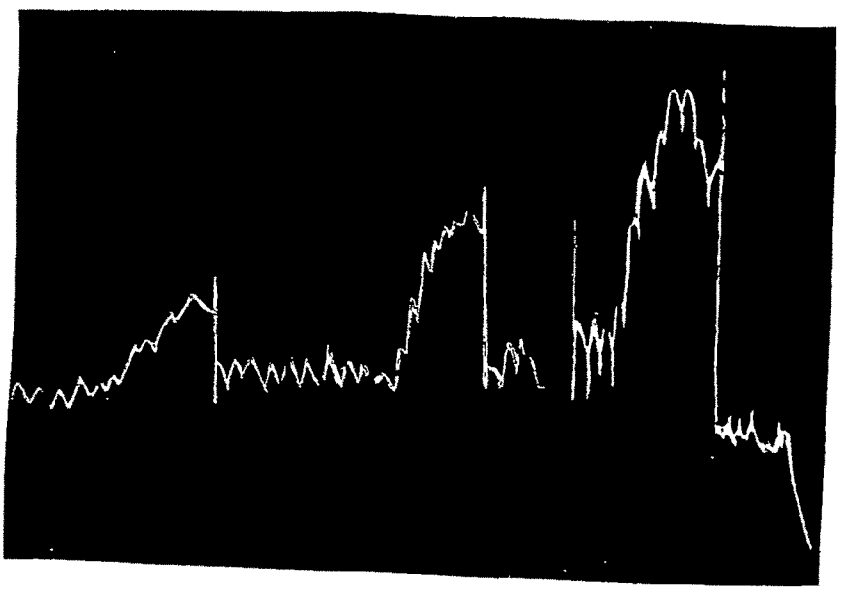
The flowers were refluxed with 100 ml of 2N HCl for 2 hours. The mixture was allowed to cool and the residue obtain after filteration was neutralized. The saponin was extracted from the residue with petroleum ether in soxhlet apparatus for 24 hours. The solvent volume was allowed to evaporate. The petroleum ether extract of Mahuda flowers evaporated on a boiling water bath, amounted to 2.56% of saponin. Earlier, Cuoney et al (1948) and Lepiovsky et al (1950) had reported the presence of crude isolate saponin in an amount of 2-5% in alfalfa (lucerne) meal. Mully and Gandhi (1977) reported that Mahuda seed meal contain 12.1% of crude isolate or 7% pure saponin. It appears that Mahuda flowers contain saponin but in a level lower to that present in Mahuda seeds, 2.56 versus 12.1% crude isolate of saponin.

In a test tube containing 100 mg of crude isolate saponin 5 ml water was added and the contents were shaken vigourously. The foam was

formed confirming that the saponin present in the flowers has foam forming property. The saponin derived on evaporation of Mahuda flowers extract had acrid smell. Earlier Nair and Benuni (1977) had also observed that 0.1% Mahuda seed saponin solution gave maximum and stable foam.

In study 2, the effects of alcoholic and water extracts of Mahuda flowers containing crude isolate saponin in various amounts, on rabbit duodenum and stomach fundus of rat, were recorded in the slow moving drum. The concentration of 0.5, 1 and 2 ml of alcoholic extract of Mahuda flowers containing 1 mg, 2 mg, and 3 mg of saponin respectively, produced contractile responses in rabbit duodenum (Figure 5.3). The concentration of saponin linearly related to the degree of contractile response as the peak produced by 3 mg of saponin content was the highest. Likewise, 0.5, 1 and 2 ml of water extract of Mahuda flowers containing 3 mg, 6 mg and 12 mg saponin respectively produced stimulant effect (contractile property) in isolated stomach fundus of rat (Figure 5.4). Comparing the contractile effects of alcoholic extract and water extracts of Mahuda flowers on rabbit duodenum and stomach fundus of rat, it was observed that the water extract caused greater contractile effects which were attributed to higher concentration of saponin in water extract of Mahuda flowers. The response of Acetylcholine (Ach) was examined in absence and presence of alcoholic extract of Mahuda flowers in rabbit duodenum (Figure 5.5). The 10 mcg of Ach produced contraction of the tissue. This contractile response of Ach was not modified by addition of 3 mg saponin of Mahuda flowers. This observation confirms the contractile property of saponin present in alcoholic extract of Mahuda flowers.

Figure 5.3 Response of alcoholic extract of Mahuda flowers in rabbit duodenum.

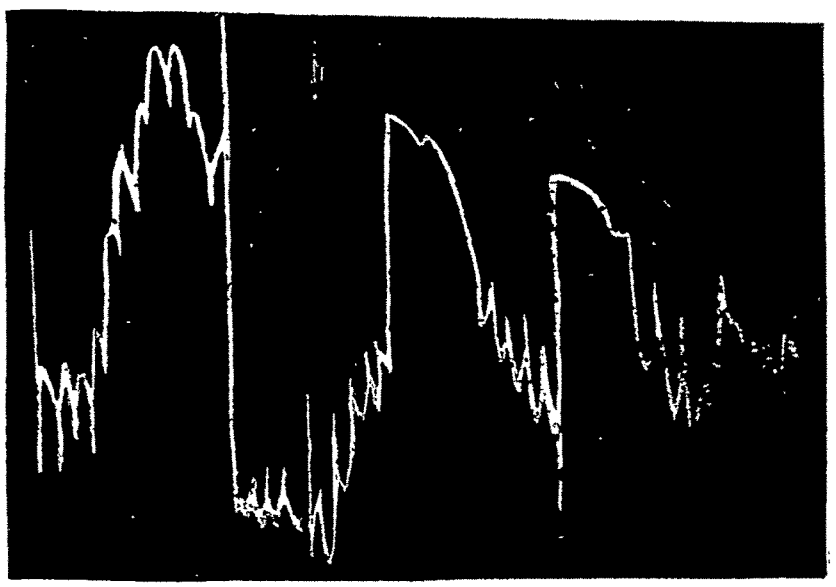


0.5 ml 1.0 ml 2.0 ml

crude saponin content

1 mg 2 mg 3 mg

Figure 5.4 Response of water extract of Mahuda flowers in rat stomach fundus.

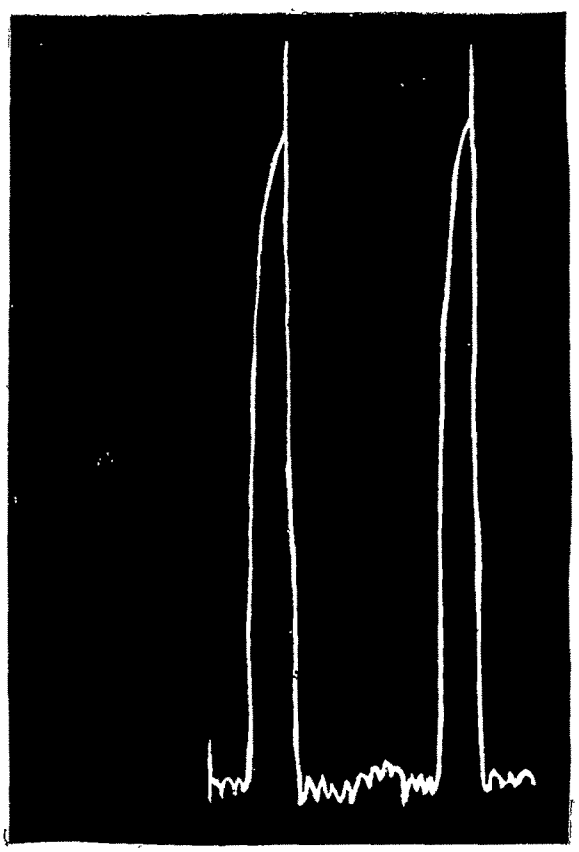


2.0 ml 1.0 ml 0.5 ml

crude saponin content

12 mg 6 mg 3 mg

Figure 5.5 Response of alcoholic extract of Mahuda flowers in presence of Acetylcholine in rabbit duodenum.



10 mcg Ach

10 mcg Ach
+ 2 ml alcoholic extract
of Mahuda flowers
containing 3 mg crude
saponin

Earlier, the pharmacological experiments conducted by Mulay and Gandhi (1977) with isolated fundus of rat and rabbit duodenum indicated that 5 mg of Mahuda seed pure saponin produced irritant/contractile activity which was followed by death of the tissue. The authors demonstrated that death of the tissue was observed from the subsequent non-reactivity of the tissue and inhibition of the normal pendular movement of the rabbit duodenum and the stomach fundus of rat. In the study 2, the in vitro pharmacological investigations conducted on rabbit duodenum and stomach fundus of the rat revealed that both alcoholic and water extracts of Mahuda flowers produced a contractile effect on the smooth muscles of the gastro-intestinal tract, although the saponin content of Mahuda flower extract was in crude form.

The study 3 was conducted to evaluate the nutritional quality of the steam treated Mahuda flowers powder incorporated into the diet at 25% level (25M30 diet). The weanling rats fed 25M30 diet for 28 days, exhibited a loss of appetite (Table 3.1). The rats ate nearly 1/3rd of the amount of food eaten by those fed 25M20 or SB diet. Consequently, the growth of the rats fed 25M30 diet was one third of those fed 25M20 diet and one fifth of those fed SB diet. These data suggest that the process of steaming followed by oven drying could not increase the efficiency of food utilization by destroying or inactivating the saponin content of the Mahuda flowers.

Table 5.2 presents mean values for weight of fresh organs expressed as percent body weight of rats fed 25M30 or 25M20 or SB diet. The liver, kidney and heart of the steam treated flower fed rats (25M30 diet) were found to be enlarged as compared to those of 25M20 or SB diet fed rats. The enlargement was more marked in the case of liver

Table 5.1 Food intake, body weight gain and FER of the weanling rats fed various diets for 28 days

	DIETS		
	Sago-Mungal gram (SP diet)	20 minutes cooled Manuka flowers at 25% level (25M20 diet)	30 minutes steamed Manuka flowers at 25% level (25M30 diet)
	MEAN ± SE		
Food intake (g)	711.25 ±13.77	704.19 ± 7.37	687.00 ±14.70
Bod, weight gain (g)	99.12 ± 4.81	62.17 ± 1.85	38.38 ± 1.26
g food needed per g weight gain	7.68 ± 0.12	9.57 ± 0.11	6.26 ± 0.12

Table 5.2 Fresh organ weight as percent body weight of rats fed various diets for 28 days.

	DIETS		
Organs	5% diet	25MP:0 diet	25MP:6 diet
	MEAN ± SE		
Liver	4.77	4.44	6.90
	±0.26	±0.11	±1.25
Intestine	4.56	4.60	5.12
	±0.10	±0.23	±0.21
Heart	0.37	0.38	0.46
	±0.02	±0.009	±0.06
Spleen	0.26	0.24	0.28
	±0.06	±0.01	±0.04
Kidney	0.71	0.80	1.19
	±0.05	±0.02	±0.17

tissue. The spleen on the other hand, of rats fed 25M20 diet weighed less than those fed 25M20 or 5M diet. The results on organ weights along with those of food intake and body weight gain suggest that it would not be safe to consume steam treated Mahuda flowers as a potential alternative food energy source.

The findings of the experiment V lead to the conclusion that Mahuda flowers contain 2.76% crude isolate saponin. Varied concentration of saponin in the alcoholic and water extracts of Mahuda flowers generated irritation of the smooth muscles of the gastro-intestinal tract. This could have been one of the reasons for decreased food intake observed in weanling rats fed 75M75 or 25M20 diet in the present investigation. Also, it became apparent that the Mahuda flower saponin could not be detoxified by simple steam treatment. It may also be that during the process of steam treatment carbohydrate from Mahuda flower might have got complexed with some component of Mahuda flower or with the toxicant present in the flower, whereby the carbohydrate became unavailable. This hypothesis is based on the fact that the growth rate of pressure cooked Mahuda flower fed rats (25M20 diet) was better than that of those fed steam treated flowers (25M20 diet). Many authors have reported various processes for detoxification of Nowrah meal by Acid hydrolysis, soxhlet extraction of the meal with ethanol, soaking of Nowrah meal with water for prolonged periods of time followed by subsequent drying, and concomitant feeding of cholesterol or phytosterols in the diet to nullify the harmful effects of saponins to some extent (Hulse 1976 Pradhan et al 1976, and Menon 1977). However, these processes of detoxification have many drawbacks including loss of solids and nutrients, and commercial impracticability.

Thus keeping the above results in mind it is essential to device the processes which can remove the Mahuda flowers saponin selectively or modify it so as to make it lose its surface activity and will render the flowers harmless. Such a process has to be economically feasible to remove and inactivate the saponins from Mahuda flowers prior to its utilization as a dietary component.