CHAPTER VII
PHYTOCHEMICAL OBSERVATIONS ON *MAESA INDICA* WALL—A CASE STUDY
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Out of 70 herbal specimen collected and screened for different pharmaco- 
cognostical studies, it was found that plant named *Maesa indica* not reported so 
far is being utilised by the people in traditional indigenous system of medicine 
for various ailments and diseases. Chopra et al (1958) mentioned its use in Ayur- 
veda as anthelmintic and anti—syphilitic. In Goa, the seeds of this plant are 
used as anthelmintic, roots are mainly used as blood purifier and also as anti- 
hypertensive, while leaves are used as blood purifier and as anthelmintic. The 
use of seeds and leaves is made from the decoction of these plant products, while 
in case of roots, the root is rubbed with milk or rice water and taken internally. 
Considering use of *Maesa indica* in traditional medicine, its availability in Goa 
and that this plant is not much worked out except few of the following reported 
works, its study was taken up. Atal et al (1978) reported that the whole plant 
excluding roots contained the tannins. Desai et al (1975) reported that the ace- 
tone extract of seeds yielded mesaconine. Aziz Ahmad and Zaman (1973) reported 
that the petroleum ether extract of leaves yielded sitosterol and ethyl acetate 
extract of leaves gave quercetin - 3 rhamnoside. However, literature survey did 
not reveal any published work on the roots of *Maesa indica*. Considering the fact 
that this plant from Goa region has not been reported so far for phytochemical 
screening, the roots of this plant were taken for the phytochemical study consi- 
dering its therapeutic value.

The roots of *Maesa indica* were collected in the month of October from
the forest of Morlem, Sattari Taluka, Goa. However the collection yield was poor due to the scarcity of the growth of this plant. Herbarium sheet of this plant was prepared and is kept in the Herbarium of Goa College of Pharmacy, Panaji-Goa. The identity was established through Botany Department of Chowgule College, Margao-Goa and it was further confirmed through Botanical Survey of India, Western Zone, Pune, Maharashtra.

The roots were cut into small chips and then dried on trays in drying oven at a temperature of 55°C until completely dried. The small chips were then placed in hammer mill and powdered. This coarse powder was then sieved through a sieve number 16 for obtaining uniformity in the size of the drug. The flavonoids were isolated as per the scheme given herewith:

<table>
<thead>
<tr>
<th>The Scheme used for the isolation of flavonoids</th>
<th>1 kg of powdered drug extracted with petroleum ether by soxhlet extractor.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plant acids, fatty &amp; waxy materials and phytosterols removed</td>
<td>Marc extracted with chloroform.</td>
</tr>
<tr>
<td>Alkaloids and other non-flavonoid substances removed</td>
<td>Marc extracted with methanol.</td>
</tr>
<tr>
<td>Flavonoid substances</td>
<td>Methanolic extract was concentrated and this concentrated extract was digested with distilled water (2 - 3 hours) two or three times and filtered through muslin.</td>
</tr>
<tr>
<td>Precipitate removed</td>
<td>Aqueous extract extracted with solvent ether in separating funnel.</td>
</tr>
<tr>
<td>Water insoluble substances.</td>
<td>Free aglycones</td>
</tr>
<tr>
<td>removed</td>
<td></td>
</tr>
</tbody>
</table>
Aqueous extract extracted with
(a) ethyl acetate.
(b) ethyl acetate : methanol (95:5)
(c) n - butanol

removed
Flavonoid glycosides.

Aqueous layer preserved.

Isolation of flavonoids and sterols

One kg. of powdered roots of *Maesa indica* was placed in a thimble made of cloth and then placed in a soxhlet extractor which was connected below to the receiving flask containing few pieces of porcelain to avoid bumping and on the top with a reducing adaptor to which a condensor was fixed. The condensor was connected to the tap water by means of a rubber tubing and the outlet of the condensor was left in the tap water basin. The whole assembly was placed on the heating mantle and extraction started using petroleum ether as the first solvent. The petroleum ether was poured from the top over the drug in the extractor till the solvent siphoned. The siphoned extract was brownish red in colour. Further more of the petroleum ether was poured so as to cover \( \frac{1}{4} \) of the volume of the drug in the extractor. The heating mantle was put on and when the solvent started boiling, the temperature was brought down and regulated, so that the constant boiling of the solvent was continued. Extraction was carried out till the siphoned extract was almost colourless. It took about 30 hours to complete the extraction and consumed \( 4 \times 3 \) litres of petroleum ether. The assembly was disassembled and extract from the flask was concentrated in vacuo and marked as concentrated extract of petroleum ether. This extract was tested for presence of flavonoids and sterols. The flavonoids were tested by Shinoda's test. It failed to show presence of flavonoids in this extract. The extract was further tested for sterols by means of Liebermann-Burchard's test, which showed positive test for sterols. Therefore, the concentrated petroleum ether extract was dried on water bath to a residue for the separation of sterols.
Next the mare was taken out from the extractor and dried in air. It was further dried in a oven 55°C. This powder was repacked in another fresh thimble and placed back in the extractor. This time, chloroform was poured in and the extraction was continued till siphoned liquid did not show colour to the extract. It required 4 x 3 litres of chloroform and 16 hours for complete extraction. This extract was concentrated and tested for presence of flavonoids and sterols. It showed absence of both substances. Therefore this extract was rejected.

The mare was again taken out, dried and repacked in extractor and extracted using methanol as solvent. Extraction continued till the colour disappeared and it took 36 hours and utilised 6 x 3 litres of methanol. This methanolic extract was concentrated in vacuo and concentrated extract was tested for presence of sterols and flavonoids. It showed absence of sterols but presence of flavonoids by the above test. Therefore, this methanolic extract was digested with distilled water for 2 to 3 hours in 250 ml water on hot water bath. It was filtered. This was repeated three times and finally all the filterates were collected together. This aqueous liquid was then concentrated to a small volume and further extracted with various organic solvents ranging from ether to ethyl acetate to ethyl acetate : methanol mixture and finally with n-butanol, with the help of separating funnel till the organic phase did not show any colour to it. The aqueous extract was first extracted with solvent ether. About 1.5 litres of ether was required. Next the aqueous liquid was extracted with ethyl acetate till completion of the extraction, it required 3 litres of ethyl acetate. It was further extracted with ethyl acetate : methanol mixture in the proportion of 95 : 5. It required 1.5 litres of this mixture. Finally, the aqueous extract was extracted with n-butanol. It required 1 litre.

All these fractionated extracts from aqueous extract were concentrated
and tested for presence of sterols and flavonoids. It was found that ether extract gave red colour with Shinoda's test, brownish green colour with ferric chloride but negative test with Molisch's reagent. It showed presence of flavonoid aglycones and absence of sterols.

Ethyl acetate and mixture of ethyl acetate : methanol extracts both showed positive test for flavonoid glycosides when tested with Shinoda's test and Molisch's test. Butanol and aqueous extracts failed to show the presence of sterol & flavonoid by chemical test. Therefore, ether extract was used to isolate flavonoid aglycones and ethyl acetate mixture extract was used to isolate flavonoid glycosides.

Examination of petroleum ether extract

The petroleum ether extract 28 g. was refluxed with 10% alcoholic potash 300 ml. for 4 hours. The alcohol was removed by distillation in vacuo frequently making up the volume with the water. The unsaponifiable matter was extracted with ether (8 x 300 ml.) and dried over anhydrous sodium sulphate. It was filtered and ether was removed by distillation and the semisolid obtained (8 g.) was dissolved in chloroform and absorbed on neutral aluminium oxide 50 g. This was poured into an alumina column prepared as below. Petroleum ether (60-80) was poured into a glass column (4 x 70 cm) plugged with cotton at the bottom. 300 g. of alumina was carefully added and the column was allowed to run for a few minutes to remove air bubbles. This was little tapped to give uniform formation of column. About 3 - 4 cm. of layer of solvent was allowed to remain on the top. The substance to be chromatographed was poured over the column without disturbing the upper layer and the elution was started with n-hexane and continued with 5%, 10%, 20% and 50% benzene in n-hexane and then benzene alone, followed by the graded mixtures of benzene & chloroform in the above order. About 400 ml. of each mixture was used for elution and 50 ml. of eluate was collected each time. Elution with chloroform was
continued up to 700 ml. The eluates up to 20% benzene in chloroform gave only uncrystallised waxy residues, while 50% benzene in chloroform and pure chloroform eluates on concentration showed a trailing spot on TLC of silica gel G when run in a solvent of chloroform : acetone (4 : 1). These eluates were concentrated to 15 ml. The detection of the spot done by spraying with 50% sulphuric acid followed by heating the plates at 120 - 140°C. Further purification of the compound was done by means of preparative thin layer chromatography (TLC), using silver nitrate coated TLC plates. 13 g. of silver nitrate was dissolved in 60 ml. of water and 30 g. of silica gel G was admixed. The plates were coated and dried in dark for 2 hours and then activated for 1 hour 110°C. In this manner, 70 plates were prepared. The solution from above containing the sterols was streaked on the plates. (0.25 ml. was streaked on each plate.) These plates were developed in a developing solvent chloroform : ether : acetic acid (97 : 2.3 : 0.5). After development, the chromoplates were dried in air. Small lengthwise portion of each plate was sprayed with 0.2% alcoholic solution of dibromofluorescein, by covering the rest of the area of the plate with a glass plate. The plate was then viewed under UV radiation of 365 nm and the two fluorescent bands were detected and marked. The corresponding bands from the rest of the plates were then scraped off and placed in two different beakers. Each band powder was then eluted with benzene by heating on hot water bath. The eluates were then filtered and the clean filtrates were evaporated to dryness. The residues so obtained from two bands were then recrystallised from methanol. The lower band of Rf value 0.86 corresponded to compound B and the upper band of Rf value 0.90 corresponded to compound A. The yield of compound A was 640 mg. and that of compound B was 110 mg. The melting points of compound A was 142°C - 144°C and while that of the compound B was 160°C - 161°C. The confirmation of the structures of these two compounds was done by spectral data analysis and preparation of derivatives.
Compound A:

Compound A: Melting point 142° - 142°. It gave red colour in Salkowski's test and a green colour in Liebermann Burchard's test. It gave a yellow colour with tetranitromethane. It was soluble in benzene and chloroform. Molecular wt from Mass is 414.

Elemental analysis: Compound A was found to have C = 83.5%, H = 12.44% & O = 4.06% against the requirement of C = 83.99%, H = 12.15% and O = 3.86%.

UV: \( \lambda_{max} \) nm 205.2. The results are shown in figure 1.

IR Bands: \( \nu_{max} \) cm\(^{-1}\) 3400 cm\(^{-1}\) (-OH) bending vibration, 3030, 1660 \( \nu_O = \nu_C \) (stretching vibration of \( \beta \)-Sitosterol.)

IR is superimposable with Aldrich catalogue No. 1494 A. The results are shown in figure 2.

Mass Spectroscopy: It gave major peaks at m/z 414 (M\(^+\)), 399 (M-Me\(^+\)), 396 (M-H\(_2\)O\(^+\)), 381 (M-Me-H\(_2\)O\(^+\)), 329 (M-85\(^+\)), 303 (M-C\(_7\)H\(_{11}\)O\(^+\)), 273 (M-Sc-H\(_2\)O\(^+\)), 231 (M-Sc-C\(_3\)H\(_6\)\(^+\)), 213 (M-Sc-C\(_3\)H\(_6\)H\(_2\)O\(^+\)). This fragmentation agrees with the literature values reported for \( \beta \)-sitosterol. The results are shown in figure 3.

Acetylation of Compound A: Compound A (50 mg.) was taken up in dry Pyridine (0.5ml) & freshly distilled acetic anhydride (3 ml.) was added to it. The mixture was refluxed for 3 hours. The mixture was kept at room temperature, overnight and then added to ice water and finally stirred. The solid obtained was filtered, dried and was crystallised from benzene as fine needles, m.p. 126° - 128°.

Benzoylation of Compound A: Compound A (50 mg.) was taken up in a dry pyridine (1.5 ml.) and benzoyl chloride was added to it. It was shaken well and left overnight. Then the mixture was poured on ice and the colourless solid formed was filtered, dried and crystallised from ethanol as shining needles m.p. 139° - 140°.

From the above analytical studies, the compound A was found to be
Figure 1

PERKIN-ELMER LAMBDA 15 UV/VIS SPECTROPHOTOMETER

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METHOD SCAN: MANUAL
β - sitosterol.

**Compound B:**

Compound B: Melting point 160° - 161°. It gave a red colour in Salkowski's test and a green colour in Liebermann Burchard's test. It gave a yellow colour with tetranitromethane. It was soluble in benzene, chloroform and petroleum ether. Molecular weight from Mass is 412.

**Elemental analysis:** Compound B was found to have C = 83.26%, H = 11.11% & O = 5.61% against the requirement of C = 84.40%, H = 11.72% & O = 3.88%.

**UV:**

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UV: | MeOH | nm 210.8
Max
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The results are shown in figure 4.

**IR Bands:**

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IR Bands | KBr | cm⁻¹
Max | 3430 cm⁻¹ (-OH), 1640 (double band)
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The results are shown in figure 5.

IR is superimposable with Aldrich catalogue No. 1494B of stigmasterol.

**NMR Signals:** (CDCl₃, 90 MHz) at δ 0.668 (S H - 18) 0.98 (S, H - 19), 1.022 (H - 21, d), 0.7 (H - 26), 0.78 (H - 29), 0.82 (H - 26), 3.45 (br, m, H - 3), 5.3 (H - 6), 5.1 (H - 22 or H - 23). The results shown in figure 6.

**Mass Spectroscopy:** Peaks at m/z 412 (M⁺), 394 (M-H₂O), 369 (M-C₃H₇)⁺, 314 (M-98)⁺, 300 (M-C₇H₁₂O)⁺, 299 (M-113)⁺, 273 (M-Sc)⁺, 272 (M-Sc-H)⁺, 255 (M-Sc-H₂O)⁺, 231 (M-Sc-C₅H₆)⁺, 229 (M-Sc-C₅H₆)⁺, 213 (M-Sc-C₅H₆-H₂O)⁺, 211 (M-Sc-C₅H₆-H₂O)⁺. This fragmentation pattern is in agreement with that described in literature for stigmasterol. The results are shown in figure 7.

**Acetylation of Compound B:** Compound B (30 mg.) was added to freshly distilled acetic anhydride (3 ml.) and fused sodium acetate (1 g.) and pyridine (1 ml.). The mixture was refluxed for 3 hours and then poured in ice cold mixture. The crude solid obtained was filtered, dried and crystallised from methanol as colourless crystals m.p. 143° - 144°.

From the above analytical studies, the compound B was found to be stigmasterol.
Figure 4.
Figure 7
Examination of ether extract:

The ether extract was concentrated in vacuo. The residue (3.4 g.) was transferred to a 100 ml. conical flask using 50 ml. of methanol. The methanolic solution was concentrated to 20 ml. and kept in refrigerator. Light yellow green crystals which separated after 3 days were filtered off, washed with little ice cold methanol and dried, (240 mg.). Thin layer chromatography (silica gel G on developing solvent, ethyl acetate : formic acid : water in proportion of 8:1:1) of this substance revealed presence of flavonoid along with other colouring matter when tested with aluminium chloride reagent. Therefore, separation of this flavonoid in the mixture was achieved by dissolving the substance in minimum quantity of methanol and subjected to silica gel column chromatography. A fine suspension of silica gel (column chromatographic grade) in ethyl acetate was prepared by stirring silica gel (200 g.) with ethyl acetate (400 ml.). This uniform sturry was added as a continuous stream to a glass column of 4 x 70 cm. size plugged at the bottom with cotton, till a height of 30 cm. silica gel was obtained. The solvent was allowed to run at the rate of 15 - 20 drops per minute. The ether residue (240 mg.) in methanol was poured over the column and eluted with ethyl acetate 300 ml. and then with gradient mixtures of ethyl acetate and methanol in proportion of 5%, 10%, 20%, 30% and 50% methanol and finally pure methanol. 300 ml. of each mixture was used and each time 50 ml. of eluate was collected and tested for presence of flavonoid. It was found that only 20% above mixtures of methanol in ethyl acetate gave similar spot when tested for TLC. Therefore, these eluates were collected together, concentrated and residue so obtained was recrystallised from methanol. Yellowish green crystals, decomposing at 347°C in the yield of 65 mg. were obtained. This compound was designated as compound C and subjected to colour test. When tested with Shinoda's test, it gave red colour and with ferric chloride reagent it gave brownish green colour. It was subjected to elemental analysis, UV IR and Mass spectroscopy. Due to paucity of the sample, it was not possible to have NMR spectra.
Compound C:

**Elemental analysis:** Compound C was found to have C = 53.60%, H = 3.63% & O = 42.77%.

**UV:**

\[
\text{MeOH} \rightarrow \text{Max} \quad \lambda_{\text{nm}} = 374.4, 252.8, 208.8, 301.2. \quad \text{The results are shown in figure 8.}
\]

**IR Bands:**

\[
\text{KBr} \rightarrow \text{Max} \quad \nu_{\text{cm}^{-1}} = \text{Hydroxyl groups at 3400, 3470, 3500 and absorption at 1655, 1620, 1560, 1510, 1490, 1460, 1440, 1360, 1330, 1280, 1240, 1225, 1200, 1170, 1120, 1090, 1040, 1025, 1000, 950, 860, 830, 800, 770, 740, 710, 670, 650.} \quad \text{The results are shown in figure 9.}
\]

**Mass Spectroscopy:** \( M^+ 270, m/e 242, 213, 196, 168, 152. \quad \text{The results are shown in figure 10.}

Since it did not match with IR of known compounds from Aldrich catalogue, based on the above data, we arrived at a tentative flavanol structure with the hydroxyl groups at 3, 6 & 8 of A ring.

**Examination of ethyl acetate and ethyl acetate : methanol (95 : 5) fractions:**

These two fractions were mixed together and concentrated to a thick solution of 15 ml. It was monitored on TLC (silica gel G) in developing solvent ethyl acetate : formic acid : water (8 : 1 : 1) and sprayed with boric acid reagent to detect the flavonoids. It showed a prominent fluorescent spot along with other non-flavonoid impurities. Therefore, this solution was poured on silica gel column prepared as below. A fine suspension of silica gel (column chromatographic grade) in ethyl acetate was prepared by stirring silica gel (100 g) with dry acetone (200 ml.). This uniform slurry was added as a continuous
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Figure 8.

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METHOD SCAN/MANUAL
stream to a glass column (2 x 45 cm) plugged at the bottom with cotton, till a height of 20 cm silica gel was obtained. The concentrated ethyl acetate extract was carefully put on the top of the column when a 2 cm height of the solvent was remaining. Elution was started first with acetone and continued with 25%, 50% ethyl acetate, pure ethyl acetate and 1%, 2% and 5% methanol in ethyl acetate. The solvent was allowed to run at the rate of 15 - 20 drops per minute. The quantity of eluate collected each time was 50 ml. and the volume of each eluant used was 300 ml. The eluates upto the ethyl acetate did not show the fluorescent spot and therefore discarded. Eluates from 1% methanol and above showed the fluorescent spot along with two other non-flavonoid spots when monitored on TLC as above. 1% methanol in ethyl acetate and above eluates on concentration gave a yellow solid (720 mg.) which was shown to consists of some impurities. Therefore, further purification of the compound was done by dissolving it in 15 ml. ethyl acetate : methanol (95 : 5) mixture and separating the compound by preparative thin layer chromatography using silica gel G as adsorbent and boric acid spray for detection. 70 plates were prepared of 500 μ thickness and the solution was streaked on the plate as in the earlier case. A small edge of the plate was utilised for boric acid spray for detection and location of the spot. The fluorescent spot occured at Rf value 0.45 free from other impurities was scrapped out from the plates and extracted from methanol. The methanolic solution was concentrated when a yellow crystalline solid of m.p. 185° - 190° in the yield of 470 mg. was obtained which was designated as compound D.

**Compound D:**

Compound D was found to have C = 47.65%, H = 5.06% & O = 47.29% against the requirement of C = 53.11, H = 4.95% & O = 41.94% Molecular weight from FDMS was reported to be 610.

\[
\text{UV: } \lambda_{\text{Max.}} \text{MeOH} \quad \text{nm} \; 375.2, 268.0, 213.2.\] The results are shown in figure 11.
IR Bands (ν) KBr Max cm\(^{-1}\). It showed broad OH at 3400 cm\(^{-1}\) and absorption peaks at 1670, 1620, 1570, 1560, 1510, 1470, 1370, 1300 and it is superimposable with Aldrich catalogue No. 906 H of rutin. The results are shown in figure 12.

Mass Spectroscopy: FIDs showed 632 (M + Na), 610 M, 464 (M - 146), m/z 302.

Hydrolysis of Compound D: 20 mg. of compound D was dissolved in 20 ml. of methanol and transferred to a 100 ml. of conical flask containing 40 ml. of 5% hydrochloric acid. The reaction mixture was refluxed for 8 hours, and then tested chromatographically for complete hydrolysis. The reaction mixture was stored in a refrigerator overnight whereby a part of the aglycone precipitated out. This precipitate was separated by filtration and the remaining aglycone in the filtrate was removed by extraction with ether. The aqueous phase of the hydrolytic solution was preserved for identification of sugars. The ether extract was evaporated on a watch glass to give a residue corresponding to aglycone. This was mixed with the precipitate earlier obtained. This aglycone was recrystallised from methanol to yield yellow crystals marked as compound E.

Compound E:
Compound E: M.P. 312 - 316° gave a bluish crimson colour with Shinoda's test and olive green colour with alcoholic ferric chloride. It was yellow under UV, becoming bright yellow with ammonia UV.

Elemental analysis: Compound E was found to have C = 51.32, H = 4.17 & O = 44.51% against the requirement of C = 59.61%, H = 3.34% & O = 37.05%.
UV : \( \lambda_{\text{Max}} \) nm 382.8, 269.2, 212.8. The results are given in figure 13.

IR Bands: \( \nu_{\text{KBr}} \) cm\(^{-1}\) It showed broad OH at 3400 cm\(^{-1}\), 1680, 1620 is superimposable with Aldrich catalogue No.906F of quercetin. The result are shown in figure 14.

Mass Spectroscopy: m/z 302 M\(^+\), 286, 270, 254, 245, 229, 212, 198, 152, 150. The results are shown in figure 15.

**Acetylation of Compound E:** Compound E (50 mg) was dissolved in freshly distilled acetic anhydride (3 ml.) and 2 drops of pyridine was added to it. The mixture was refluxed for two hours and left overnight at room temperature. The product was crystallised from ethanol to yield colourless needles m.p. 198° - 200°.

**Complete methylation of Compound E:** Compound E (50 mg.) was refluxed with freshly distilled dimethyl sulphate (2 ml.) and anhydrous potassium carbonate (5 g.) in dry acetone for 48 hours and then the mixture was poured into ice water. The precipitate was collected, washed with water and dried. It was recrystallised from methanol to yield colourless needles m.p. 152° - 153°.

From the above analytical studies, the compound E was found to be quercetin.

**Examination of aqueous phase of hydrolytic solution of Compound D:**
This aqueous phase was utilised for identification of the sugars. The aqueous hydrolytic solution free of aglycone was acidic and therefore was neutralised by ion exchange resin (Dowex - 2). Sufficient quantity of resin was taken
in a 250 ml. beaker. 100 ml. of 4% of sodium hydroxide was added and the mixture was kept stirring with a mechanical stirrer for 40 minutes. The resin was filtered through muslin and washed several times with distilled water till the washings were neutral to red litmus. 100 ml. of 4% hydrochloric acid was added to the resin and again stirred for 40 minutes, filtered and muslin was washed till the washings were neutral to blue litmus. The resin was then ready for use.

This regenerated ion exchange resin was added to the acidic hydrolytic solution (containing sugars) in portions with continuous stirring till the neutralisation was complete as tested with litmus. The resin was filtered off on a filter paper using a buchner funnel and washed with hot water 2 to 3 times to remove the adhering sugars completely. The aqueous filtrates were combined and concentrated in chinadish over a hot water bath. The residue obtained was dissolved in 1 ml. of pyridine and spotted on a chromatographic paper (whatman No.1) along with solutions of known sugars rhamnose & glucose. 5μl of a 5% sugar solution was applied. The chromatogram was developed for 16 hours (overnight) with butanol : pyridine : water (7:4:3) as solvent system. The developed chromatogram was allowed to dry in air and then sprayed with aniline pthalate reagent. After heating the chromatogram in oven at 110°C for 10 minutes, the unknown sugars were identified by direct comparison of its Rf values with those of known sugars. Two spots were seen, one a dark brown spot corresponding to D-Glucose Rf value 0.43 and other as pink spot corresponding to L-Rhamnose Rf value 0.70. Thus the sugar were identified as D-glucose and L-rhamnose. Thus from the analytical studies, Compound D was identified as Rutin.