CHAPTER 2

MATERIAL AND METHODS
A. Introduction:

All living world is organic in origin. Chemical science has made its progress much before the biochemical science. Today, voluminous literatures are available on methods of organic and biochemical analysis. And, added to this, in the recent years, many sophisticated techniques have evolved. Out of these a simple technique of detecting organic compounds of plant and animal origin is chromatography. Amongst the chromatographical techniques, paper chromatography, thin layer chromatography are largely employed in qualitative detection of various organic compounds. Even quantification can be done provided the colour intensity of the compound in question on the chromato-grams has a direct relation with the amount of compounds separated. However, these days, many sophisticated techniques for instance, High Performance Liquid Chromatography (HPLC) are available to precisely quantify the chemical compounds. Coupling of such chromatographic technique with mass spectrometry, both quantitative as well as qualitative characterisation is possible. Moreover even TLC technique can be refined to High Performance Thin Layer Chromatography (HPTLC) for the precise identification of the compounds.

As such most plant compounds are well documented in literature and they fall into vast majority of genera such as the alkoloids, the steroids, the terpines, the phenolics, the tanins, the flavonoids, the glycosides etc. They are both heterocyclic as well as homocyclic. Thanks to the early workers such as Seshadri, Harborne, Mabry, Vyankatraman, Pridham, Swain and many others whose sustained efforts to identify these chemical compounds of plant origin not only led to the characterisation and identification of these
natural products but also in providing firm pedestal to the pharmacology and indigenous medical science such as Ayurveda.

It is true that in present investigation various plants are employed to prepare the recipe. However, our sense of reasoning directed to primarily scan the basic chemical principle synergistically working in the composite recipe prepared using various parts of number of plants.

The IR spectro-photometric scanning provides a clue in a broader way where to look into. Such type of study has been carried out initially before characterising compounds used in the preparation of the recipe.

B. Methods of IR Spectrophotometric Study

1) Extraction:

The extraction of flavone and flavanol glycosides is essentially based on the method of Harbone Mabry & Mabry (1975). The dried samples of each of the plant material in the finely pounded form was extracted with 80% ethanol after keeping the sample in the solvent for nearly four hours. After cold extraction twice, it was filtered through Whatmann Filter paper No.1 under vacuum. This ethanolic extract was then condensed on waterbath, till the volume is reduced to one to two ml. This alcoholic concentrate of the extract was later subjected for preparative methods of Infra Red Spectroscopy.

2. IR spectrophotometric Analysis:

Since the plant samples contains large number of organic compounds of medicinal importance, it is essential to scan each sample of the plant
materials for active ingredients which are found predominantly. Therefore, IR spectrophotometric study was carried out.

IR spectra of the plant sample was taken on IR spectrophotometre, Perkin Elemer 377 by courtesy of Dr. S.V. Badchikar, Atic Industries, Atul, Gujrat.

3. IR Sample Preparation

The procedure followed for IR sample preparation that for solid samples is of routine nature, described by Harborne (1973): Here one mg dried extract sample is mixed thoroughly with 200 mg of dried potassium bromide. The resulting mixture is pressed under pressure of 10 tons to form the pellets. The pellets of each of the sample so formed are kept on the pellet holder. The infra red spectral scanning is done, by putting the pellet holder in between the prism and the source. The scanning is done for the wavelength 4000 to 400 cm\(^{-1}\). The spectra printout is taken for examination and study for the functional groups.

C. TLC Analysis

Extract of the samples taken for IR spectrophotometric scanning was used even for TLC characterisation.

1. Preparation of the TLC Plate:

For thin layer chromatographic analysis 20x20 cm size Corning glass plate previously washed several times, both by detergent and degreasing agent and subsequently dried in the oven were used. The silica gel G containing 13% calcium sulphate 1/2 \(\text{H}_2\text{O}\) as a binder was used. 10 gm of the Kieselgur-G was taken in the beaker, to which 50 ml distilled water was
added. It was quickly and vigorously mixed by stirring with the glass rod and poured at the bottom of the plate. With the help of applicator it was evenly spread on the plate and dried in the oven at 110°C. The total thickness of the plate was maintained to 0.25 mm. So dried plates were used for the TLC separation of flavones and flavonoids.

2. Loading and running of the plates:

To accomplish this on each of the plate samples of thirteen plant material were spotted with the help of micro pippete. The quantity of each sample spotted on the plate was 10 microlitre. After drying the plates the plates were run in a corning glass chamber previously saturated with the fumes of the solvent. The solvent used for the separation is mixture of Butanol, Acetic acid and water, mixed in the proportion of 4:1:5. It is essential to mention here that the chemical used are of high purity analytical ones. The plates were run for 6 hours for better separation. After the solvent front reached the other end before it is over run the plates are removed and dried in an oven at 34°C. After drying the compounds separated were identified on the basis of colour development under UV and UV plus ammonia and Rf values as per Harborne (1973).

D. High Performance Thin Layer Chromatography Analysis:

Each of the plant samples used in the recipe was further subjected to HPTLC analysis to get the precise separation as well as quantification. This analysis was carried out on HPTLC model Camag (Switzerland) with the Scanner TLC Scanner II (Ver.3.15) by courtesy of Dr. S.V. Badachikar Atic Industries Ltd., Atul, Valsad, Gujrat.
Procedure -

The sample preparation technique was same as described earlier. The plates used for HPTLC are Kieselgel 60 F 254 precoated aluminium plate of 20x20 cm size, the thickness of the coat 0.2 mm. The plates are of E Merk, Darmstadt.

3. Separation Technique:

The extract of the samples are spotted on the plate with the help of nanomat III with 5 microlitre capillary. Plates are dried in the oven for five minutes after spotting. They were run in the glass chamber in the same way as described earlier in the 3 different solvent systems. They were dried for 5 minutes in an oven before elution. The dried plates are used for scanning with the help of TLC scanner II at the wavelength 254 nm. The qualitative estimation of the compounds present in the sample is based on the Rf values and the colour developed under UV & UV plus ammonia. The solvent system used for each of the spotted plates are based on the polarity of the compounds. Group A consist of Benzene:Acetone (90:10) for weakly polar phenolic derivatives such as Kaempferol, Coumarin, Quercetin etc.. Group B - Toulene: Chloroform: Acetone (40:25:35) for medium polar phenolic compounds such as Phenol carboxyl acid, hydroxy coumarins, flavones, aglycones etc. and Group C for ethylacetate: Butanon: Formic acid : water (50:30:10:10): for strongly polar compounds such as Kenferol, Afzelin, vitexin, Robinin etc.

4. Quantitative Analysis:

Quantitative analysis of the compound could be done with the help of TLC scanner model Camag (Switzerland). The plates were kept under the
scanner and slowly moved in the direction of the front. The scanner scanned the spots developed under UV densitometrically. The quantitative determination has been made by microprocessor attached software.

C. Chromatographic Analysis of Flavanols and Flavones:

Not all compounds are extractable in any one solvent. This is because the wide range of organic compounds such as falvonols, flavones that exist, differ in the properties. Harbone (1973) has suggested or rather recommended different techniques of extraction and analysis. As some of these compounds are extractable in 70 % ethanol, which is widely used solvent for extraction so the 2 M HCl. Moreover while extracting in some solvent system such as ethanol traces of undesirable compounds like lipids also are elicited. Such compounds often interfere in the proper separation of compounds on the chromatogram. It is essential to remove such compounds before the sample is taken for chromatographic analysis.

Based on the above, while extracting and analysing suspected chemical principles of our interest, analytical techniques have been variously blended and employed. nonethe-less they all fall into the basic tenets of analytical techniques recommended in standard books (Harborne,1973; Harborne, Mabry, 1975).

D. Paper chromatography technique:

For paper chromatographic separation of various groups, both acid hydrolysis and extraction as well as extraction in 70% ethanol are used.
1. Acid hydrolysis

This has been carried out as per Harborne (1973). Plant samples used in the preparation of recipe were first hydrolysed with 2 M HCl for 30-40 min. at 100°C. The cooled solution is extracted twice with ethyl acetate. The ethyl acetate extract was coloured possibly because the colour has formed from leucoanthocyanidin during acid treatment and hence the aqueous extract is further heated to remove the last traces of ethyl acetate and reextracted with small volume of amyl alcohol. The ethyl acetate extract is concentrated to dryness and taken up into 1-2 drops of ethanol and aliquots chromatographed one dimensionally in 5 solvents:

a) Forestal - Prepared by mixing acetic acid, conc. HCl and water in proportion 10:3:30.

b) 50% Acetic acid - Prepared by mixing 50 ml fuming acetic acid with 50 ml of distilled water. This solution becomes aqueous acetic acid.

c) BAW - (n butanol, acetic acid and water): This is prepared by taking n butanol, acetic acid and water sequentially in the proportion of 4:1:5 in a separating funnel. Holding the bottom of the funnel firm it is given a whirling movement till the 3 thoroughly mixed and then was allowed to settle. After some time 2 layers separated out. The lower layer was run off and the top layer was retained for chromatographic separation.

d) PhOH water - The phenol was first distilled and it was poured in distilled water till it reached the saturation.

e) Water - Glass distilled water was taken, and the combined extract taken to dryness and the residue taken up in small quantity of ethanol for
chromatography. This brings not only the flavonols and flavones but also some glycosides as well as the aglycones.

2. Technique of Ethanolic Extraction -

This method has been followed as per Harbone Mabry & Mabry (1975). The method described by them refers specifically to the flavones and flavonol glycosides as distinct from their aglycones. Here the dried plant materials were first powdered up and are extracted with 70% ethanol. They were first crushed in mortar with pestle and kept overnight for release of those compounds in the solvent which otherwise take time. They were filtered through Whatmann No.1 filter paper under vacuo. After repeated extraction with ethanol the extracts were taken in the Erlenmayer flask and to that 20 ml petroleum ether was added. This procedure has said to have removed the lipid if any based on partitioning principle. Total mixture was then taken into separating funnel and given a whirling movement. After some time the petroleum ether layer separated out on the top in which all the lipid content of the sample moved. The lower aqueous ethanolic layer was collected in a beaker and dried on water-bath in vacuo. After condensing to 1/4th the volume the extracts were centrifused to remove any sedimenting material that existed. The supernatant was taken for paper chromatography as well as the separation. This procedure has said to have removed total lipid impurities of the crude plant extract before paper chromatography or TLC is attempted which otherwise caused considerable streaking during the separation on chromatogram (Harborne & Williams, 1975).
3. Separation of Flavonols & Flavones:

It is necessary to mention here in the present investigation that the study is mainly concentrated on separation and detection of flavonols and flavones because in the entire recipe the medicinal principles concocted, either individually or wholly, there is any probability of containing anthocynidic. It is evident that anthocynidine group mainly include the colouring compounds predominantly found in floral parts and to certain extent in the colour foliage; and amongst the eleven plant parts used none in this sense are coloured.

Harborne (1973) while dealing with these compounds has separately treated flavonols and flavones from that of Anthocynins despite the fact that these compounds are very widely distributed in plants; especially the former both as co-pigments to anthocynins in flower petals and also in leaves. Nonetheless they occurred in glycosidic combination. According to him there is considerable range of flavanols present in the plant. Over 70 different glycosides of quercetin alone have been described and moreover these have great importance in pharmaceutical industry.

Flavones and flavonols sharply differ by missing 3 hydroxy substitution in the former. This difference in structure distinguishes them in their chemical properties by absorbing UV radiation, chromatographic mobility and colour reactions. Simple flavones can be distinguished with flavonols on these bases.

a) Glycosyl flavones: Yet there is another group called glycosylflavónes which is c-glycosides and are not soluble in ethyl acetate and remain in aqueous layer after hydrolytic treatment. They differ from flavone
aglycones in this property. Therefore, it becomes essential to separate glycosyl flavones if present either by adding ammonium sulphate into ethyl acetate extract and salt them out or extract the aqueous layer with amylalcohol so that they are transferred into that. Another feature of glycosyl flavones is that they undergo isomerisation during acid hydrolysis and give two spots on chromatogram. Under the influence of acid the pyrol link of the flavone is known to open up and when it recloses a mixture of 8-C glucoside and 6-C glucoside is present.

b) Biflavonyls - These compounds are highly polar and migrate to the front in most solvents on paper. They can be separated by TLC on silica gel using toluene:ethyl formate: formic acid (5:4:1) solvent. There are other methods of separating more obstinate biflavonysl mixture on silica gel, for instance using benzene:pyridine formic acid (30:9:5) mixture.

c) glycosides

These are conveniently separated by paper chromatographically by solvent system BAW. Alternatively instead of PC, TLC method is also employed. One of the well known TLC technique and far more difficult separation is Polyamide absorbant with a solvent mixture chloroform : Methanol : butanone (9 : 4 : 2).

4. Chromatographic technique :

a) Separation of flavons, Flavonols and Flavonones - 

For separation flavanols and flavons in each of the plant material used as well as the recipe concocted, acid hydrolysed extract of each of the plant material described earlier are used. For paper chromatography
18x12 cm Whatman filter No.1 sheet was cut in a machine direction and 5 microlitre each of the extracts were loaded at 1 cm distance from the bottom with the help of micropipette. The plates were dried and run in a glass chamber. The solvent used is forestol. The solvent forestol is prepared by mixing conc. HCl:glacial acetic acid: water (3:30:10). The freshly prepared forestol initially poured in the chamber before the chromatogram paper was rested into that, so that the chamber could get saturated with the fumes of forestal. Depending upon atmospheric humidity, temperature, saturation coefficient of the chamber it took about 6-8 hours for complete running. After having run the chromatogram they were dried in the air and taken for detection of the spots in a U.V. chamber and subsequently under the influence of UV and ammonia fumes. Based on the Rf value and the colour, the spots were identified.

b) Separation of Flavones and Flavonones by BAW -

Parallel to that of forestal solvent system the chromatograms were also run in BAW. After having loaded the sample extract in the same way as described above, the chromatograms were run in solvent system Butanol:Acetic acid: Water. This was prepared by mixing n-butanol:glacial acetic acid and water in the proportion of (4:1:5). The mixture were first mixed sequentially in a separating funnel and shaken up by giving whirling movement. It was allowed to stand and the lower layer was run down and the upper layer was taken as a solvent. The chromatographic chamber was first saturated by the solvent so that proper and final separation was accomplished. It normally takes 6 to 8 hours for the movement. The chromatograms were then dried in the laboratory in the room temperature. The spots separated were identified, first under UV in a UV chamber and
subsequently against ammonic fumes in the UV chamber. Based on the Rf values and colour of the spots under UV and UV plus ammonia the compounds were identified.

c) Separation of the compounds in 50% aqueous acetic acid:

Paper chromatograms after loading the samples in the way described earlier were run in a solvent system 50% acetic acid. Here glacial acetic acid freshly opened bottle was diluted to 50% by adding distilled water. This freshly prepared solvent was first poured in the chamber to attain the saturation. Later on the chromatogram was placed. The solvent was allowed to run for 6.8 hrs to accomplish better separation. After running the full length the chromatograms were taken and air dried in the laboratory and were then held under UV in the UV chamber. Subsequently the spots were also identified under ammonia fumes in the UV chamber. Based on the Rf values and the colour of the each spot under UV and UV plus ammonia the compound were identified.

d) Separation under Phenol Water:

The procedure followed for paper chromatographic separation for flavonoid compounds is same as above, but the solvent chosen here is phenol water. High grade commercial phenol was first distilled. To the known quantity of water phenol was added till it is saturated and this solvent was taken in chromatographic chamber. After running the chromato-gram with the full length, they were removed and air dried in the laboratory. Next day they were held under UV lamp in a UV chamber for the separation of the spots. The colour of the spot under UV was first noted. Subsequently the chamber was saturated with ammonia fumes and
colour of the spots were also noted under UV plus ammonia. Both based on the colour and the Rf value in the solvent system the compounds were identified.

e) Chromatographic separation with plain water -

The loaded chromatograms were held in the chromato-graphic chamber containing distilled water enough to dip the bottom of the paper. So run chromatograms where water is chosen as a solvent system where air dried and the spots were identified under UV and subsequently UV plus ammonia. Based on the calculation in UV and UV plus ammonia, as well as the Rf value the compounds were identified.

f) Separation of glycosyl flavones

To separate out glycosyl flavones from flavones and aglycones the aqueous layer after hydrolitic treatment, is further extracted with small amount of amyl alcohol. This tends to glycosyl flavones to move into the n- amyl alcohol layer which remains above. The lower layer from the separating funnel is run down and the upper layer is taken. Alternatively the whole procedure is carried out in a small graduated centrifuge tube where from the upper layer of the amyl alcohol is pipetted out.

g) Chromatographic separation of glycosyl flavones -

For separation of glycosyl flavone compounds three solvent systems were chosen - water, BAW and aqueous phenol. The method of preparation of solvents BAW and phenol water and the method of running chromatogram is same as described early.
h) Separation of Bioflavonyls -

These compounds are ordinarily eluted in acid digestion and aqueous extraction and hence they do not need any separate treatment. Since the compounds migrate on the front in most solvents on paper TLC technique is recommended.

TLC plates used here are of E Merk Darmstadt: The samples were loaded with the help of micropipettes and each of the plates were run in solvents water, BAW and saturated PhOH. The method used is same as of P.C. The spots were identified under UV in UV chamber first and the colour were noted. Subsequently they were exposed to ammonia fumes in the UV chamber. Based on the colour of the spots under UV and UV plus ammonia they were identified.

i) Separation of O-glycosides

These are easily separated by PC from the acid digested aqueous extract. Extract of each of the sample were loaded on the chromatogram as described earlier and each of the loaded chromatogram was run in a solvent system BAW and water. The method of preparation of solvent is same as that described earlier.

j) Polyamide separation of O-glycoside -

Polyamide TLC were loaded with the sample and run in a solvent mixture chloroform:methanol:butanone (ethyl methyl ketone) mixed in the proportion of (9:4:2). The rest of the procedure is same as described earlier.
k) Separation of minor flavonoids -

Minor flavonoids like that of chalcones aurones, flavonones, dihydrochalcones and isoflavones, paper chromatography is the best technique to use. Paper chromatographic technique is used as per previous one. The solvent system used here is BAW (4:1:5).

l) Separation of Flavonones -

Detection and separation of flavonones is done in a bit different way. It has got a separate colour test. The alcoholic solution is taken in a test tube and a pinch of magnesium is added into it. Then it is treated with concentrated hydrochloric acid. This is a reduction method.
Preparatory Techniques

a) **Extraction**:

The extraction of flavone and flavonol glycosides is essentially based on the method of Stahl (1969).

The dried samples of each of the plant material in the finely powdered form was quantitatively extracted, sequentially in (1) Petroleum ether, (2) Chloroform, (3) Diethylether, (4) Acetone and (5) Methanol.

The samples were taken in the Petroleum ether and crushed in a mortar with pestle and filtered through Whatman filter No.1 under vacuo. This facilitates removal of the fat. Subsequently the samples were extracted in each of the following solvents chloroform diethyl ether, acetone and methanol.

The chloroform extract contained chiefly compounds following into the category methylated flavones many furfuralaminos, daphnoretin etc. While the ether extracted aglycones and some glycosides. Most of the glycosides are then found into acetones and methanol.

All these plant extracts are quantitatively transferred to a penicillin vial and stored in a secured way and further analysed for various compounds employing TLC techniques.
Experimental conditions for TLC:

For the TLC separation of various compounds predominantly flavonoids, three absorbents were used: (1) Cellulose, (2) Silica gel and (3) Polyamide.

All these plates of different adsorbents were available with E. Merk Darmstadt, Germany. The same are being employed for thin layer chromatographic analysis. All these TLC plates of 20x20 cm size were coated with 0.25 mm thick respective adsorbent spread over aluminium plates.

Loading the sample:

5 µl quantity each of the 12 plant extracts, plus one of the recipe were mechanically loaded on the TLC plate with the help of nanomat III attached with the micropippete. They were dried and run in the following solvents:

1) Benzene acetone (90:10),
2) Petroleum ether (high boiling point): benzene methanol Butanone (50+40+5+5).

These above solvents were chosen to achieve the separation of slightly polar phenol derivatives on both silica gel and polyamide layers. This is included in Group A.

In the Group B medium polar compounds such as phenolics, hydrocoumarins, flavone-aglycones are included. Their separation has been achieved on cellulose, silica gel and polyamide layers. The solvents employed are -
1) For cellulose - Chloroform:Acetic acid: Water (50+45+5)

2) For silica gel - Toluene:Chloroform:Acetone (40+25+35)

3) For polyamide - Benzene:Butanone:Methanol (60+20+20)

Group C:

Strongly polar compounds, which include flavone glycosides. Their separation has been achieved on the plates silica gel, polyamide and cellulose.

1) For Silica gel - Ethyl acetate:Butanone:Fomic acid; water
   (50+30+10+10).

2) Polyamide - Benzene : Methanol : Butanone (60+20+20).

3) Cellulose : Partridge mixture.

Partridge Mixture:

The Partridge mixture is the upper phase of the mixture of Butanol : Acetic acid : Water in the proportion 40+10+50. This mixture yields all glycosides excellently with the sharp resolution.

Purpose of selecting different adsorbents in TLC:

It is a long standing experience that certain adsorbents have a special value in the separation of plant phenolic derivatives, predominant being cellulose, silica gel and polyamide. Besides these three many others polyacrylamide and ion exchanger like umberlite are also recommended. However, the general experience has shown that the former three help to achieve to large extent the separation of wide varieties of phenolics
glycosides, flavonoids etc. with a sharp distinction in polarity. In general, experience has also shown that the silica gel is the best adsorbent for group A i.e. less polar compounds. A polyamide layer is the best for the separation of compounds containing free phenol groups and the corresponding glycosides (flavone compounds). On the other hand cellulose layers have been employed in particular for separating mixtures of substances with high proportion of glycoside units.

It is felt appropriate to write a few words about each of the adsorbents used in this experiment to achieve better chromatographic separation.

**Cellulose:**

The cellulose plates used in the present experiments are all of Merk Darmstadt and the solvent systems employed are as follows:

1) Chloroform : Acetic acid : Water. 50+40+10

2) Butanol: Acetic acid : Water . 40+10+50

Upper phase as described earlier, is used.

**Silica gel -**

The silica gel plate is also of Merk Darmstadt. The capacity of the silica gel is substantially higher and because of its purely inorganic nature it goes very well with all the substances and also with aggressive reagents. The choice of the solvent is governed primarily by the polarity of the substance mixture to be separated, the more polar the mixture, the more polar the solvent must be, so that it is more powerful eluent. The most

Depending upon the possible compounds existing in the plant material.

The solvent No. (2), (6) and (7) of the above have been employed.

**Polyamide**:

According to Egger (1969) the application of polyamide to the chromatography of phenolic compounds of plant constituents has considerable advantages. It is well known that powerful hydrogen bonds are formed between phenolic hydroxyl groups and amide groups. The formation of definite addition of compounds depends on these strong subsidiary valency forces, for instance between phenol and urea in 2:1 ratio.

The chromatography of phenolic compounds on polyamide has been extensively used for isolation and structure elucidation of various naturally occurring materials including pigments of beets, various ommochromosomes, carboxylic acids and numerous nitro compounds. Quinones are irreversibly retained on polyamide as a result of free amino groups in the latter. A good separation of quinoid and phenolic compound is possible on acetylated-polyamide.
Polyamide powder is prepared by dissolving commercial perlon powder e.g. ultramid BM-2-K-228 in 35% HCl and precipitating it with methanol or water 1:1 stirring energetically. The precipitate is filtered off and dried 10 gm powder is suspended in 100 ml methanol for coating 5 c.m. plates.

Polyamide layers are most simply prepared from powder specially made commercially for TLC following the instructions given above (Endres 1969).

However in the present investigations Merk Darmstadt make, ready plates of 0.25 mm thick are used.

The Polar Solvents:

Following rules for TLC, a polyamide layer used in water, alcohol mixture is applied.

i) The more isolated phenolic hydroxyl group a substance possess, the more strongly it is retained on the layer.

ii) o-dihydroxy and vicinol trihydroxy group in the molecule influence. The chromatographic behaviour to about the same extent as a single hydroxyl group.

iii) Reaction of a hydroxyl group to form a glycosidic linkage greatly increases the Rf value of the compound. The value depends however, on the nature of the sugar. The compound is not retained on the polyamide adsorbent when it no longer contains a free phenolic hydroxyl group.

iv) Phenols adhere most strongly when water is the solvent. An elutropic series thus be established.
Table 2.1 Data A data base on the chemical principles of plants used in the recipe.

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<th>Sr.</th>
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| 7. *Cyprus Rotundus* Nagarmotha  
*cyperaceae* | Rhizome | Two new piperidine alkaloids - pipernonaline and pipерundecalidine isolated from fruits.  
Cyperene-1 and cyperene-2 isolated from tubers  
Patchoulenone, mp 52°.  
A new sequiterpene ketone - mustakene bp. 128°/1 mm - from essential oil, has same skeleton as copaene.  
Isolation, structure and absolute configuration of cyperotundone (Cyperenone) mp 46°, from tubers  
Cyperolone, mp 41°, from tubers  
27 Compounds separated from essential oil by GLC and four of these capadiene, bp. 130°/1 mm, epoxyguaiene, bp 102°/1 mm, rotundone, bp 128°/1 mm and cyperolone, bp. 120°/0.1 mm - characterised.  
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<td>9)</td>
<td><em>Terminalia belerica</em></td>
<td>Fruit</td>
<td>Kernel oil had purgative action and its prolonged use was well tolerated in mice. The oil was bland but its hydrolysed product, like castor oil, was irritant. A new cardiac glycoside—bellericanin isolated which yielded glucose and galactose (2:1). Fruit extract produced fall in blood pressure of rat at 70mg/kg. It produced significant (P-0.02) increase of bile secretion at dose of 3 mg/kg in dogs. β-Sitosterol, gallic acid, ellagic acid, ethyl gallate, galloyl glucose and chebulagic acid isolated from fruits.</td>
<td>Indian J. Med. Res. 1969, 57, 103. J. Indian Chem. Soc. 1968, 45, 913. J. Res. Indian Med. 1975, 10, 27. Indian J. Chem. 1970, 8, 1047.</td>
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<td>Sr.</td>
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<td>Isolation and structure elucidation of two aliphatic tetrols -1, 2, 3, 4-tetrol and eicosan-1, 2, 3, 4-tetrol - from gum resin.</td>
<td>Tetrahedron 1973, 52, 221.</td>
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<td>Linoleic, oleic, steoric and palmitic acids, sitosterol, stigmasterol, cholesterol, campesterol and α-spina-sterol identified in seed oil.</td>
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<td>Euphorbiceae</td>
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<td>Preparation of phyllembic acid salts and structure studies.</td>
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<td>Seed fat contained linoleic acid (64.8%) and closely resembled linseed oil.</td>
<td>J. Oil Technol. Assoc. India, 1973, 5, 8; Chem. Abstr. 1973, 78, 156602 a.</td>
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<td>Prunus cerasoides</td>
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<td>Indole acetic acid and four other auxins-α1, α3, α4 and α5- detected in immature fruit; two growth inhibitors - R1 and R2 also detected in fruit.</td>
<td>New Phytol. 1981, 88, 53; Chem.Abstr. 1981, 95, 147283 q.</td>
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<td>Naringenin-4'-methylether-7-xyloside isolated from seeds.</td>
<td>Phytochemistry 1982, 21, 1464.</td>
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