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Extraction, Isolation and Characterization Screening of *Coccinia grandis*

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ABSTRACT

Herbal medicine is the oldest form of medicine known to mankind. It was the mainstay of many early civilization and still the most widely practiced form of medicine in the world today. Medicinal plants produce a diverse range of bioactive molecules, making them rich sources of different types of medicines. *Coccinia grandis* whole plant part is used in Siddha, Ayurveda, Unani, traditional and ethano medicinal system. The present study is focus on the phytochemical analysis of ethanolic extract of *Coccinia grandis* aerial parts. Qualitative and quantitative phytochemical analysis of this species extract confirms the presence of various phytochemicals like alkaloids, glycosides, phenolic compounds and steroids. Purification of isolated compounds has been done and characterization of isolated compounds performed through spectral techniques like IR, NMR, UV, Mass spectrum. UV spectra have shown maximum absorption due to substitution of functional group in compound A. The IR spectrum of compound A showed absorption bands characteristics of flavanol indicating the presence of hydroxyl and tinsoporin functionalities at 3449, 1375 and 1079 cm^{-1} respectively, indication of double bond were represented by 1689 cm^{-1}. Peak at 1375 cm^{-1} represented C-H bending whereas NMR was also characteristic of phytotaxanol, exhibiting the hydroxyl proton signal at δ 5.21as a multiplet, which helped us to characterize this compound as taxaterone, tinsoporin A and B respectively

Keywords- *Coccinia grandis*, Phytochemical screening, Isolation, Mass spectrum

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INTRODUCTION

The World Health Organization (WHO) estimates that about 80% of the population living in the developing countries relies almost exclusively on traditional medicine for their primary health care needs. In almost all the traditional medicine, the medicinal plant plays a major role and constitutes the backbone of traditional medicine. Medicinal plants are a source of great economic value in the Indian subcontinent and India has one of the richest plant medical traditions in the world. India has a tradition that is of remarkable contemporary relevance for ensuring health security to the millions. Nature has bestowed on India a rich botanical wealth and a large number of diverse types of plants grow in different parts of the country. India is rich in all the 3 levels of biodiversity, namely species diversity, genetic diversity and habitat diversity. India has 2.4% of world's area with 8% of global biodiversity[1, 2]. The Cucurbitaceae family is commonly known as gourd, melon and pumpkin family. The family of *Coccinia grandis* is cucurbitaceae, comprises 960 species. The family is predominantly distributed around the tropics. Most of the plants in cucurbitaceae family are annual vines .Coccinia includes 29 additional species and they are found only in tropical Africa. *Coccinia grandis* is used by humans mostly as a food crop in several countries like Australia, Asia, Caribbean, and the southern United States, Pacific Islands. This plant used in traditional and ethanomedicinal in many countries for the treatment of human diseases[3]. So the present study includes qualitative and quantitative phytochemical screening of *Coccinia grandis* aerial part.

MATERIALS AND METHODS

Collection and identification

Plant was collected from nearby area of Pune, Maharashtra, India, during the months of August and September and specimen deposited for Taxonomic and ethno medicinal identification to Botanical Survey of India, Pune, Maharashtra.

Preparation of plant material

The aerial parts of plant were shade dried, reduced to coarse powder with the help of grinder and stored in airtight container. 1 kg of powdered drugs were packed in soxhlet apparatus and continuously extracted with petroleum ether to defatting the drug. Petroleum ether was removed from the powdered defatted drug, which was then extracted with ethanol (95%). The alcoholic extract thus obtained was further fractioned with hexane, chloroform and ethanol. The solvents were removed from each extract and fraction by

distillation and the last traces of solvent being removed under reduced pressure. The extracts and fractions were weighed and their % value was recorded which is shown in table 1 and also the physical appearance, color and odor was evaluated and recorded and thereafter, extracts were stored in refrigerator for further experimental work.[4,5,6]

Physical Parameter[7]

Ash Values

Determination of total ash value

Accurately weighed about 3 g of air dried powdered drug was taken in a tarred silica crucible and incinerated by gradually increasing the temperature to make it dull red hot until free from carbon. Cooled and weighed, repeated for constant value. Then the percentage of total ash was calculated with reference to the air dried drug.

Determination of acid insoluble ash value

The ash obtained as directed under total ash value was boiled with 25 ml of 2N HCL for 5 minutes. The insoluble matter was collected on an ash less filter paper, washed with hot water, ignited and weighed, then calculated the percentage of acid insoluble ash with reference to the air dried drug.

Determination of water soluble ash value

The total ash obtained was boiled with 25 ml of water for 5 minutes. The insoluble matter was collected on an ash less filter paper, washed with hot water and ignited for 15 minutes at a temperature not exceeding 450°C. The weight of insoluble matter was subtracted from the weight of total ash. The difference in weight represents the water soluble ash. The percentage of water soluble ash was calculated with reference to the air dried drug. All the ash values were calculated and recorded in table 2

Extractive Values

Extractive values of crude drugs are useful for their evaluation, especially when the constituents of a drug cannot be readily estimated by any other means. Further, these values indicate the nature of the constituents present in a crude drug.

Determination of Alcohol Soluble Extractive Value

10g of the air-dried coarse powder of *Coccinia grandis* macerated with 100 ml of 90% ethanol in a closed flask for 24 hours, shaking frequently during the first 6 hours and allowing standing for 18 hours. Thereafter, it was filtered rapidly taking precautions against loss of the solvent. Out of that filtrate, 25 ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish, dried at 105°C and weighed. The percentage of ethanol soluble extractive value was calculated with reference to the air-dried drugs

Determination of Water Soluble Extractive Value

Weigh accurately the 10 g of coarsely powdered drugs and macerate it with 100 ml of water in a closed flask for 24 hours, shaking frequently during the first 6 hours and allowing standing for 18 hours. Thereafter, it was filtered rapidly taking precautions against loss of the solvent. Then 25 ml of the filtrate was evaporated to dryness in a tarred flat-bottomed shallow dish, dried at 105°C and weighed. The percentage of water soluble extractive was calculated with reference to the air dried drugs.

The percentage of water soluble extractive and alcohol soluble extractive were calculated and recorded in the table 2.

Loss on Drying

About 1.5 g, of powdered drug was weighed accurately in a porcelain dish which was previously dried at 105°C in hot air oven to constant weight and then weighed. From the difference in weight, the percentage loss of drying with reference to the air dried substance was calculated and listed in table 2

Phytochemical Screening [7, 8]

Qualitative examination of phytoconstituents:

Test for Alkaloids

Dragendorff's Test: To 1 g of the extract, add 1 ml of Dragendorff's reagent (Potassium Bismuth iodide solution). An orange-red precipitate indicates the presence of alkaloids.

Ethanol extract, hexane fraction, chloroform fraction and ethanol fraction give the orange- red precipitate.

Mayer's Test: To 1 g of the extract, add 1 ml of Mayer's reagent (Potassium mercuric iodide solution). Whitish yellow or cream colored precipitate indicates the presence of alkaloids.

Ethanol extract, hexane fraction, chloroform fraction and ethanol fraction give the cream colored precipitate.

Wagner's Test: To 1 g of the extract, add 2 ml of Wagner's reagent (Iodine in Potassium Iodide), Formation of reddish brown precipitate indicates the presence of alkaloids.

Ethanol extract, hexane fraction, chloroform fraction and ethanol fraction give the reddish brown precipitate.

Test for Carbohydrates

Molisch's Test: To 2g of the extract, add 1ml of α-napthol solution, add concentrated sulphuric acid through the side of the test tube. Purple or reddish violet color at the junction of the two liquids reveals the presence of Carbohydrates.

Ethanol extract and ethanol fraction give purple color at the junction of the two liquids.

Fehling's Test: To 1gm of the extract, add equal quantities of Fehling solution A and B, upon heating formation of a brick red precipitate indicates the presence of sugars.

Ethanol extract and ethanol fraction give brick red precipitate.

Test for Glycosides

Legal Test: Dissolve the extract in pyridine and add sodium nitroprusside solution to make it alkaline. The formation of pink red to red color shows the presence of glycosides.

Ethanol extract and its three fractions do not give pink red to red color.

Baljet Test: To 1gm of the test extract, add 1gm of sodium picrate solution and the yellow to orange color reveals the presence of glycosides.

Ethanol extract and its three fractions do not give yellow to orange color.

Test for Tannins and Phenolic Compounds

1. Take the little quantity of test solution and mixed with basic lead acetate solution. Formation of white precipitates indicates the presence of tannins. Ethanol extract, ethanol fraction, hexane fraction, and chloroform fraction give white precipitates.

2. To 1gm of the extract, add ferric chloride solution, formation of a dark blue or greenish black color product shows the presence of tannins. Ethanol extract, ethanol fraction, hexane fraction, and chloroform fraction give dark blue color.

3. The little quantity of test extract is treated with Potassium ferric cyanide and ammonia solution. A deep red color indicates the presence of tannins .Ethanol extract; ethanol fraction, hexane fraction, and chloroform fraction give deep red color.

Test for Steroids

Libermann-Burchard Test: 1g of the test substance was dissolved in a few drops of chloroform, 3ml of acetic anhydride, 3ml of glacial acetic acid were added, warmed and cooled under the tap and drops of concentrated sulphuric acid were added along the sides of the test tube. Appearance of bluish-green color shows the presence of sterols.

Ethanol extract, ethanol fraction, hexane fraction, and chloroform fraction give bluish-green color.

Test for Triterpenoids

Noller's Test: Dissolve two or three granules of tin metal in 2 ml thionyl chloride solution. Then add 1gm of the extract into test tube and warm, the formation of pink color indicates the presence of triterpenoids. Ethanol extract, ethanol fraction and chloroform fraction give pink color.

Test for Flavonoid

Little quantity of extract is treated with amyl alcohol, sodium acetate and ferric chloride. A yellow color solution formed, disappears on addition of an acid indicates the presence of Flavonoid.

Ethanol extract, ethanol fraction and hexane fraction yellow color solution formed, disappears on addition of an acid.

Test for Saponins

Take small quantity of alcoholic and aqueous extract separately and add 20 ml of distilled water and shake in a graduated cylinder for 15 minutes lengthwise. A 1cm layer of foam indicates the presence of saponins.

Quantitative Determination of the Chemical Constituents:

Total phenols Determination

The total content of phenols in the crude ethanol extract was determined using a modified Folin-Ciocalteu colorimetric method with Gallic acid as a standard. The extract solution in DMSO (700 µL) was transferred to a 10 mL volumetric flask, the Folin-Ciocalteu reagent (400 µL) was added and after 3 min, each flask was made up to the mark with sodium carbonate (Na_2CO_3) solution (75 g/L). After 2 hours, the suspension was centrifuged (5000 r.p.m., 5 min) and the absorbance of the solution was measured at 760 nm. The total phenolic content was expressed as a Gallic acid equivalent (GAE) in g/100 g of dry extract. Data are reported as mean ± SD for three replicates. The total phenol was calculated and recorded in table 4.

Total alkaloid determination

5 g of the sample was weighed and transfer into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 h. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed. The total alkaloid was calculated and recorded in table 4.

Total tannin determination

500 mg of the sample was weighed into a 50 ml plastic bottle. 50 ml of distilled water was added and shaken for 1 h in a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to the mark. Then 5 ml of the filtered was pipetted out into a test tube and mixed with 2 ml of 0.1 M Ferric chloride in 0.1 N HCL and 0.008 M potassium Ferro cyanide. The absorbance was measured at 120 nm within 10 min. The total tannin was calculated and recorded in table 4.

Total flavonoid determination

10 g of the plant sample was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through what man filter paper No 42 (125 mm). The filtrate was later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight. The total flavonoid was calculated and recorded in table 4

Thin layer chromatography (TLC) [8]

Preparation of plates: Slurry of silica gel G and distilled water was prepared in a pestle with continuous triturating with mortar. The slurry was spread evenly on clean grease free glass plates. The plates were dried in air and thereafter heated in oven at 110 ^{0}C for about 30 minutes to activate them[23].

Preparation of samples: Approximately 10 mg of material was dissolved in respective solvents and was used for spotting on TLC plates.

Application of samples on TLC plates: Samples were applied on the TLC plates with the help of a capillary tube at a distance of about 0.5 cm from the developing solution. The solvent from the plate was removed by air-drying and position of the spot was marked.

Saturation of TLC chamber: The inner wall of the chamber was lined with filter paper on three sides, the solvent system was poured up to a height of about 1 cm from the base, grease was applied on the rim of the chamber and it was covered with a glass plate. The chamber was allowed to stand for about 30 minutes and by that time the filter paper inside the chamber was completely drenched by the solvent system, making the chamber completely and evenly saturated with solvent system.

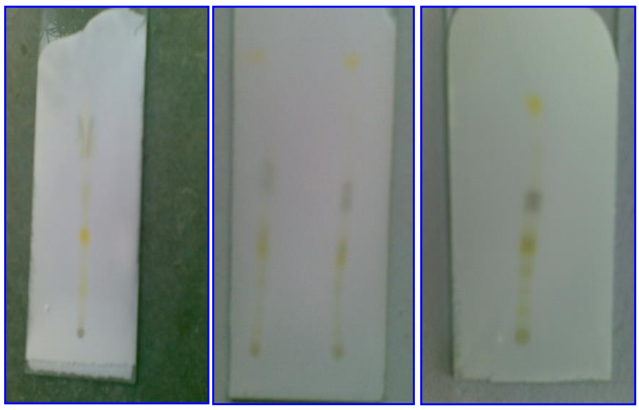
Development of TLC plates: The plates were placed vertically into a solvent vapor saturated TLC chamber and allowed to develop till the mobile phase had moved about 80% from the spotting line; the plate was removed from the developing chamber and dried.

Detection of TLC plates: The plate were exposed to iodine vapor and observed.

The TLC profile was examined to determine variation in band size and color intensity between the ethanolic extract and its three fraction .The observation indicate that variations are present amongst ethanolic extract, ethanolic fraction, Hexane fraction and chloroform fraction. The TLC study was done by using different solvent system for each fraction separately and the compounds were detected by sun

light, in iodine vapours, vanillin sulfuric acid reagent and long UV and all the results are presented in table 5 and 6

Optimization of solvent system: hexane soluble, chloroform soluble and ethanol soluble fractions were analyzed by TLC. These fractions constituted of mainly nonvolatile mixtures of compounds. The visualizations were aided by either observing the TLC under an UV lamp or by exposing the developed TLC plates to iodine vapor. The TLC was repeatedly improved by changing the solvent systems until a system that gave the best separation was obtained.



A B C

Fig 1: TLC studies of Ethanolic fraction [A], hexane fraction [B], and chloroform fraction [C] for *Coccinia grandis*

RESULT AND DISCUSSION

Table 1: Characteristics of *Coccinia grandis* extract and fraction

Extract/ fraction	Yield(%w/w)	Physical appearance	Color	Odor
Pet. Ether extract	6.8	Syrupy liquid	Dark green	Pungent aromatic
Ethanol extract	18.8	Syrupy mass	Greenish black	Aromatic
Hexane fraction	6.8	Syrupy mass	Greenish black	Aromatic
Chloroform fraction	5.0	Semisolid mass	Dark green	Characteristically aromatic
Ethanolic fraction	7.2	Semisolid mass	Greenish black	Aromatic

Table: 2 Physical parameters of *Coccinia grandis* powder

Studied parameters	Observation (% w/w)
Loss on drying	6.9
Total ash value	5.2
Acid insoluble ash value	4.3
Water soluble ash value	3.1
Alcohol extractive value	16.7
Water extractive value	6.7

Table 3: phytochemical presents in extract and fraction of *Coccania grandis*

Test for	Ethanolic extract	Hexane fraction	Chloroform fraction	Ethanolic fraction
Alkaloids	+	-	+	+
Carbohydrates	-	-	-	+
Glycosides	+	-	-	+
Phenols and tannins	+	-	-	+
Steroids	+	+	+	+
Terpenoids	+	-	+	+
Saponins	-	-	-	-
Flavonoid	+	+	-	+

(+) = present, (-) = absent

Table 4: Percentage of phytoconstituents present in *Coccinia grandis*

Constituents presents	Quantity of phytoconstituents in (%)
Alkaloids	10.32
Phenols	3.33
Flavonoids	8.32
Tannin	2.68

Table 5: TLC Studies of Ethanolic extract and Ethanolic fraction of *Coccinia grandis*

Fraction/ Extract	Solvent system	No of spots	TLC profile	
			R_f value	Color
Ethanolic extract	Toluene: Ethyl acetate: Metenol:Water (7:6:5:2)	9	0.94;0.90;0.81; 0.74;0.69;0.61; 0.59;0.45;0.38	Dark green, green, green, faint green ,pale green, yellow , light yellow, light green, brown
Ethanolic Fraction	Ethyl acetate: methanol: toluene: water (5:4:6:5)	5	0.90;0.86;0.73; 0.54;0.32	Dark green, green, light green, light yellow, brown

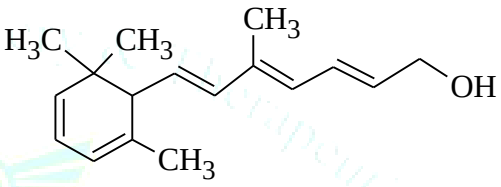
Table 6: TLC studies of Hexane fraction and chloroform fraction of *Coccinia grandis*

Fraction/ Extract	Solvent system	No of spots	TLC profile	
			R_f value	Color
Hexane fraction	Hexane: DCM:Ethyl acetate: Methanol (10:5:2:3)	6	0.76;047;0.41 0.29;0.27;0.22	Dark yellow, Dark brown, Dark brown, Very light green. Light yellow, light brown
Chloroform fraction	Hexane: Ethyl acetate: methanol (10:3:7)	5	0.70;0.64;0.58; 0.47;0.39	Light green, Very light green, Light brown , light yellow , light brown

Spectral Analysis and Structural Elucidation

It was tried to purify the compounds, which were obtained by employing column chromatography (CC) and preparative thin layer chromatography (PTLC), and by re-crystallizing them in different solvents. The compounds were weighed and there melting point and solubility was determined. The identification of a molecule was done through the interpretation of the data obtained from spectroscopic analysis. Tools for vital structure elucidation of natural products are Fourier transform infrared spectroscopy (FTIR) Agilent Cary 630 FTIR spectrometer , mass spectrometer (MS) Agilent 6520 (Q-TOF) , and nuclear magnetic resonance spectroscopy (NMR) Bruker II Advance 400 FT-NMR . With these tools the structures of most natural products can be determined. [9, 10]

Compound A



IUPAC Name: 2E,4E,6E)-5-methyl-7-(2,6,6-trimethylcyclohexa-2,4-dien-1-yl)hepta-2,4,6-trien-1-ol

Table 7: Spectral data of Compound -A

IR (KBr) ν_{max}cm $^{-1}$	^{1}H NMR (in ppm) δ	Melting Point range	Ultra Violet Wave Length	Mass Spectra
3683 (alcohols, phenols) Stretch 3397 (OH stretch, H-bonded) 1635 (aromatics) 1521 (C-C stetch) 849 (C-H stretch)	0.675 s br (methyl) 1.49 s br (ethyl) 1.6 s br(ethyl) 1.9 s br (Propyl) 2.18d (J= 12.0 Hz) 5.21d (J= 4.0, 2.0 Hz) (ether)	230-232 ^{0}C	λ max= 259 Absorbance=0.813	Base Pea207. M+ Peak242.2

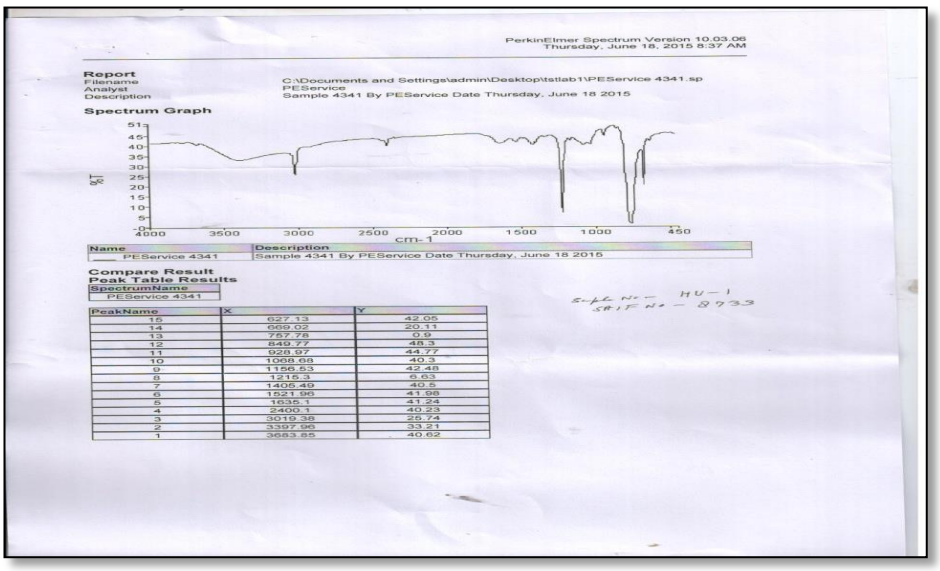


Fig 2 – IR spectrum of compound A

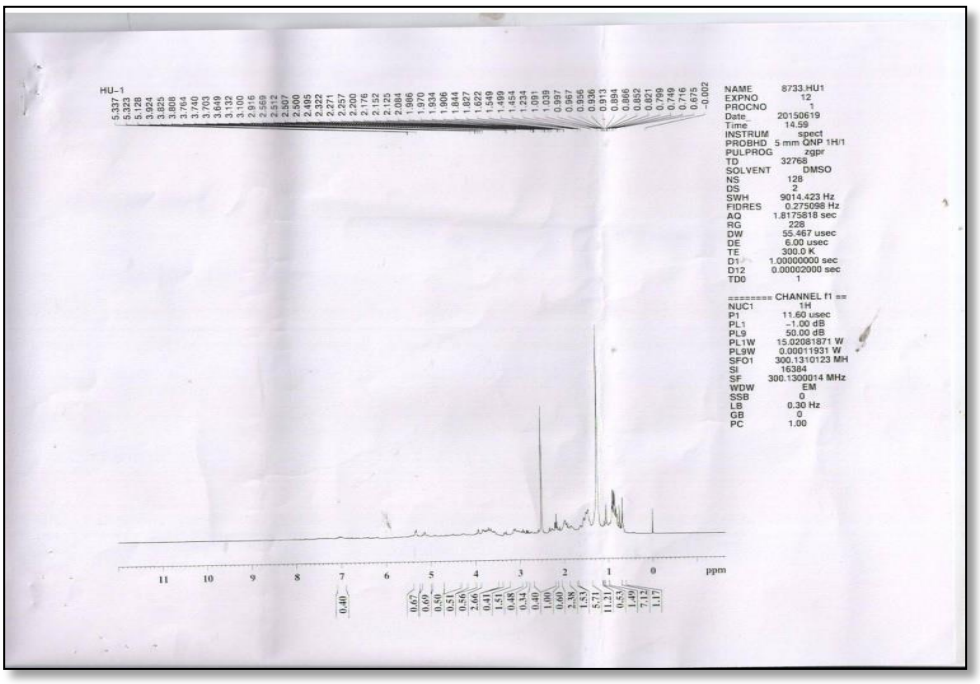


Fig 3 - ^{1}H-NMR spectra of compound A

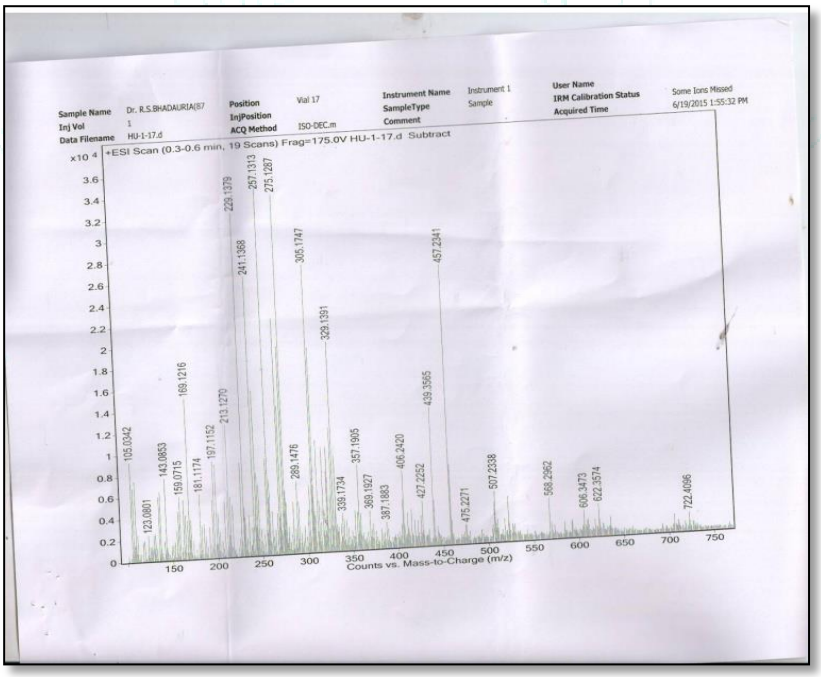
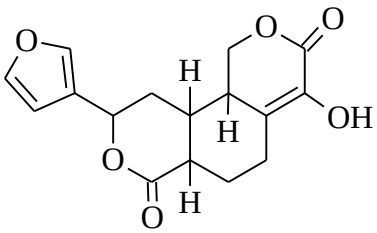


Fig.-4 Mass spectrometry of compound A

Compound B



IUPAC Name: 9-(furan-3-yl)-4-hydroxy-1,5,6,6a,9,10,10a,10b-octahydro-3H,7H-pyrano[3,4-f]isochromene-3,7-dione

Table 8: Spectral data of Compound-B

IR (KBr) ν_{max}cm $^{-1}$	^{1}H NMR (in ppm) δ	Melting Point range	Ultra Violet Wave Length	Mass Spectra
3458 (alcohols, phenols) Stretch 3487 (OH stretch, H-bonded) 2100(aldehydes) 1720 (aromatics) 1422 (C-C stetch) 1215 (alkyl halides) 1099 (carboxylic acids)	0.687 s br (methyl) 4.55 br (hydroxyl) 1.7 s S (ethyl) 2.3 s S(ethyl) 2.18d (J= 12.0 Hz) 5.21d (J= 4.0, 2.0 Hz) (ether)	287-288 ^{0}C	λ max= 220 Absorbance=0.621	Base Pea278. M+ Peak304.7

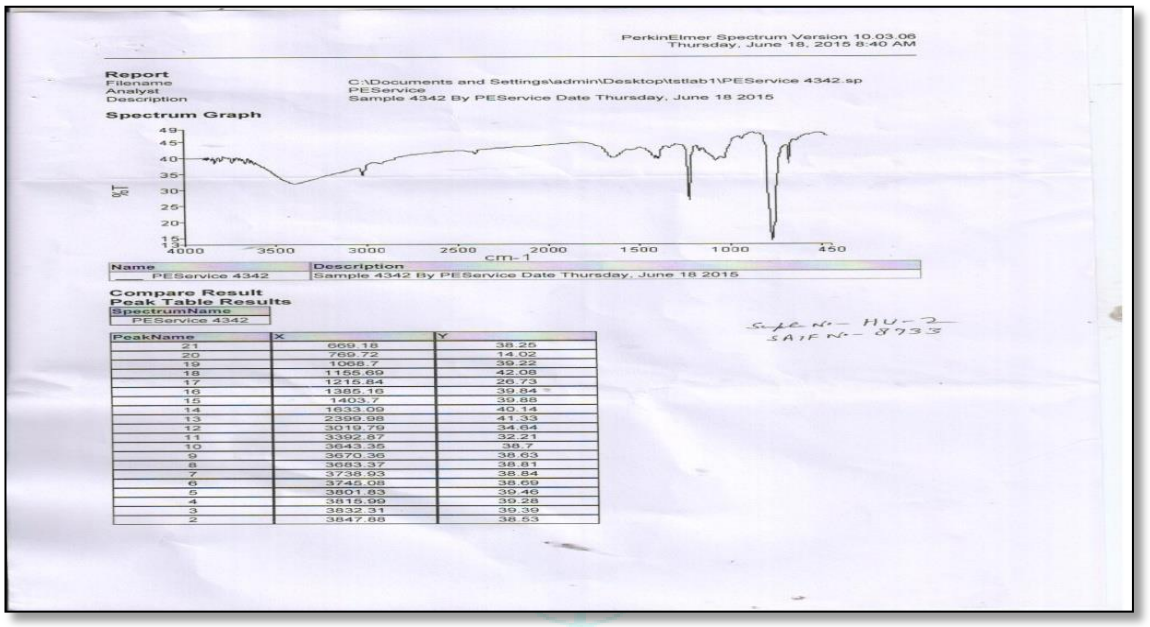


Fig 5 – IR spectrum of compound B

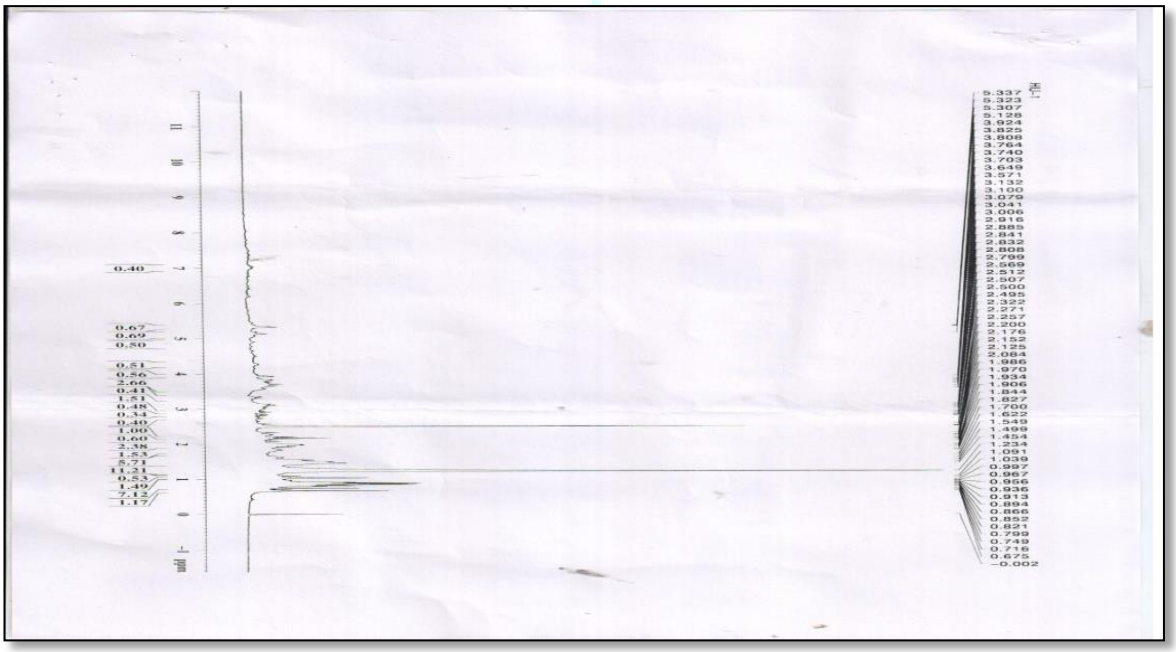


Fig 6- ^{1}H-NMR spectra of compound B

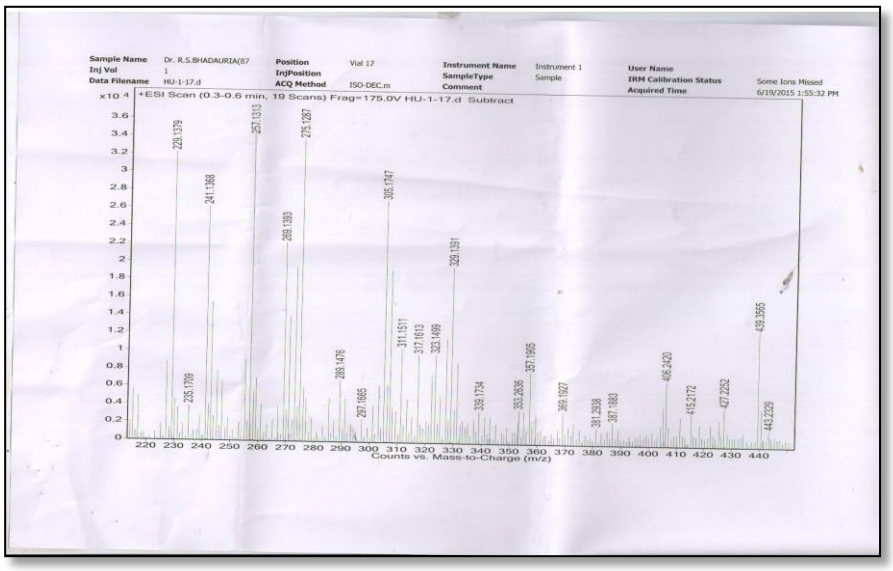


Fig.-7 Mass spectrometry of compound B

CONCLUSION

The plant *Coccinia grandis* have been found to be source of medicinal agents based on their use in traditional medicines. In present work Purification of isolated compounds has been done and characterization of isolated compounds has been done through spectral techniques like IR, NMR, UV, Mass spectrum. UV spectra have shown maximum absorption due to substitution of functional group in compound A. The IR spectrum of compounds A showed absorption bands characteristics of flavone indicating the presence of hydroxyl and tinsoporin functionalities at 3449, 1375 and 1079 cm^{-1} respectively, indication of double bond were represented by 1689 cm^{-1}. peak at 1375 cm^{-1} represented C-H bending whereas NMR spectra was also characteristic of phytotaxanol, exhibiting the hydroxyl proton signal at δ 5.21as a multiplet, which helped us to characterize this compound as taxaterone, tinsoporin compound A and B respectively.

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