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# Phytochemical Characterization of *Hylocereus polyrhizus* Rind Extracts

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***Authors' contributions***

*This work was carried out in collaboration between all authors. Author SY designed the study, author SMS performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors JJ and ARAS managed the analyses of the study. Author RSS managed the literature searches. All authors read and approved the final manuscript.*

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## ABSTRACT

The purpose of this study was to identify the phytochemical components of rind extracts from *Hylocereus polyrhizus*. Two distinct solvents were used in the maceration process to create the extracts. Following a preliminary phytochemical study of the extracts, the principal bioactive substances, including tannins, flavonoids, and alkaloids, were quantitatively identified. Using LC-MS and HPTLC, the *H. polyrhizus* rind extracts are phytochemically characterized. Chemical profiling of the extracts, retention factor of the separated bands, and constituent percentage area were all determined using HPTLC. By using LC-MS, about 28 phytochemical components and compounds were examined. A sizable concentration of bioactive chemicals in *H. polyrhizus* suggests that the plant has pharmacological properties that include neurological, anticancer, antidiabetic, antioxidant, and cardioprotective properties.

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*Keywords: Hylocereus polyrhizus; phytochemicals; LC-MS; HPTLC.*

## 1. INTRODUCTION

The Hylocereus genus, sometimes referred to as pitaya or dragon fruit, is a genus of climbing cacti that has gained popularity throughout the world for its aesthetic qualities and as a profitable fruit crop [1,2]. This unique superfruit, which is found in India and is a member of the Cactaceae family, is becoming more and more well-liked in rural as well as urban areas due to its eye-catching colour, mouthwatering flavour, and many nutritional and therapeutic benefits [3]. It's considered a great natural antioxidant and micronutrient source [4,5]. Vitamins A, C, and E, alkaloids, terpenoids, flavonoids, thiamine, niacin, pyridoxine, cobalamin, phenolics, and beta-carotene are among the components found in dragon fruit peel. Numerous pharmacological actions, including antifertility, anticancer, antidiabetic, cardioprotective, and neuroprotective ones, have been documented [6]. The fruit was given the moniker "dragon fruit" because of its overlaying green fins covering brilliant red skin. This fruit is also known by the name's pitahaya, dragon pearl fruit, strawberry pear, night-blooming cereus, and Cinderella plant [7]. Dragon fruit can be utilized as functional components, natural colorants, environmentally friendly and active packaging, edible films, for the creation of photoprotective products, and as additions in food and nutraceutical products.

## 2. MATERIALS AND METHODS

### 2.1 Plant Sample Collection and Preparation

In March 2024, *H. polyrhizus* was taken from the lulu hypermarket in Trivandrum. Dr. E. A. Siril, a professor and head of the botany department at the University of Kerala in Kariavattom, Trivandrum, recognized and verified the plant material. The Department of Botany of the University of Kerala maintains a voucher specimen, KUBH 11388. After peeling and chopping the rind into tiny pieces, it was dried and dehydrated for seven days in the shade. An ultrafine pulveriser was used to powder the dehydrated samples, which were then sealed in a vial and kept in a freezer at -18°C.

### 2.2 Extract Preparation

The powdered samples were extracted by maceration:

a. Ethanolic extract – 140 grams of powdered *H. polyrhizus* rind plant material was weighed, suspended in 1L of ethanol, and shaken periodically for three consecutive days at room temperature. After filtering through Whatman filter paper number 1, the suspension was concentrated at lower pressure at a temperature range of 25 to 60 degrees Celsius. Until it was needed, the extract was stored in a refrigerator at 4°C. The ethanol extract's percentage yield was then determined to be 9.7% w/w and classified as HPEE.
b. Aqueous extract - 140 grams of powdered *H. polyrhizus* rind plant material was weighed, suspended in 1L of water, and shaken periodically for a week at room temperature. After filtering through Whatman filter paper number 1, the suspension was concentrated at lower pressure at a temperature range of 25 to 60 degrees Celsius. Until it was needed, the extract was stored in a refrigerator at 4°C. After that, the aqueous extract was given the designation HPAE, and 51.65% w/w was determined to be its percentage yield.

### 2.3 Qualitative Determination

**Phytochemical screening:** The following techniques were used to identify the extracts' tannins, alkaloids, flavonoids, phenols, saponins, terpenoids, glycosides, proteins, and carbohydrates using qualitative phytochemical testing [8,9,10,11].

**1. Tannins**

- Gelatin test- Add an aqueous solution containing 1% gelatin and 10% NACL to the test extract. The precipitate was a buff white colour.
- Phenazone test- We boiled and cooled ten millilitres of the test sample extract along with sodium acid phosphate. After the mixture of solution has been filtered, 2% phenazone solution is added. The precipitate was thick and pigmented.

**2. Alkaloids**

- Dragendroff test-2ml of the test extract and dilute hydrochloric acid (0.2 ml) were taken

in a test tube. After adding 1 ml of Dragendroff reagent, orange brown precipitate was obtained.

- Mayer s test-when 2ml of the test extract and 1ml of Mayer s reagent were taken in a test tube, creamy white precipitate was obtained.

**3. Saponins**

- About 1ml of the extract is mixed vigorously with 5 ml of distilled water, formation of foam indicates the presence of saponins.

**4. Glycosides**

- Borntrager's test- add 5–10 ml of dilute HCl boil to the test extract on water bath for 10 min and filter. Filtrate was extracted with CCl4/ benzene and add equal amount of ammonia solution to filtrate and shake. Formation of pink or red color in ammoniacal layer indicates the presence of anthraquinone moiety.

**5. Flavonoids**

- Shinoda test-About 1ml of each extract and a few magnesium turnings were added to test tube. A few drops of concentrated hydrochloric acid were carefully along the walls of the tube. Appearance of red color indicates the presence of flavonoids.

**6. Coumarins**

- 1 ml of 10% NAOH solution was added to 1 ml of the extract. Yellow color formation confirmed the presence of coumarins.

**7. Carbohydrates**

- Add 2 drops of Molisch's reagent to the 2 ml of the test extract in a test tube. Add concentrated sulphuric acid along the sides of the test tube, a purple-colored ring is formed at the interface of two layers.

**8. Triterpenoids**

- About 2ml of trichloro acetic acid were added to 1ml of the test extract. Red colored precipitate indicates the presence of triterpenoids.

**9. Proteins**

- Biuret test- to 2ml of the test extract, add 1ml of 40%NaOH and few drops of copper sulphate. A purple violet color was obtained.

**10. Phenols**

- Ferric chloride test-Add 1-2 drops of fecl3 to 2ml of the test extract. Blue, green color indicates presence of phenols.

**11. Steroids**

- A few drops of acetic anhydride are added to the extracts and the formation of violet to blue to green in some samples indicates the presence of steroids.

**12. Terpenoids**

- Salkowski test-5ml of the test Extract was mixed with 2ml of chloroform, and concentrated sulphuric acid was carefully added to form a layer. A reddish-brown color at the interface indicates the presence of terpenoids.

## 2.4 Quantitative Determination

**Quantitative determination of tannin in extracts of *H. Polyrhizus*:** Tannins in the extracts were determined using Trease and Evans method [12]. About 0.5g prepared samples was weighed and added into a conical flask where 50ml of distilled water was added. The contents were filtered after mixing for 1 hour. About 5ml of the filtrate and 5ml of 0.1%tannic acid were added respectively. 5ml of distilled water added to 5 volumetric flasks of 50ml capacity was used as blank, and at 20°C in a water bath. Then the three flasks containing text extract, standard and blank were filled up with distilled water up to the 50ml mark. The flasks were incubated for 1 hour 30 minutes. The concentration was determined at 760 nm using UV-Visible Spectrophotometer.

**Quantitative determination of flavonoid in extracts of *H. Polyrhizus*:** Flavonoid in samples was determined by the method of Obadoni and Ochuko [13]. About 10g of sample and 100ml of 80%aqeous methanol was added into a 250ml conical flask. the mixture was properly mixed for three hours on an electronic shaker. Into a weighed beaker the mixture was filtered and on a water bath, it was evaporated to dryness and weighed again until a constant weight was obtained.

**Quantitative Determination of Alkaloids in Extracts of *H. Polyrhizus*:** The method of Harborne [14] was used to determine alkaloids in

the samples. 5g of powered sample and 200 ml of 10% acetic acid in ethanol was added into a 250ml beaker. At room temperature, the mixture was allowed to stand for 4 hours while covered, and then filtered. Alkaloid was precipitated after drop wise addition of concentrated aqueous ammonium solution into the collected filtrate. It was then concentrated to solid by evaporation on a water bath. the alkaloid precipitated was placed in a filter paper, it was weighed as W1, and content was washed by 1% ammonium solution, dried at 80oC in the oven. After drying and cooling, the filter paper was weighed and residue gave W2. The formula below was used to calculate alkaloids in the samples and was expressed in percentage weight.

## 2.5 Thin Layer chromatography

The test extracts were applied on pre-coated TLC plates by using capillary tubes and developed in a TLC chamber using suitable mobile phase. Different solvent systems of different polarities were prepared, and TLC studies were carried out to determine the suitable solvent system capable of showing better resolution [15]. The best separation of the compound was found in the system of n-butanol: glacial acetic acid: water (4:1:1). The developed TLC plates were air dried and observed under ultraviolet light (UV) at both 254 nm and 366 nm. Then they were placed in a hot air oven for 1 min for the development of color in separated bands. The movement of the solvent was expressed by its retention factor (Rf).

Retention Factor ($R_f$) =distance travelled by solute/distance travelled by solvent.

After drying the plates, they were exposed to iodine vapors by placing them in a chamber that was saturated with iodine vapors. All plates were visualized directly after drying and with the help of UV at 254 nm and 366 nm in UV chamber.

## 2.6 High Performance Thin Layer Chromatography

The given sample extracts (aqueous and water) were dissolved in methanol: water mixture.10 microliter of each sample were applied on 10 x10cm silica gel 60 F 254 TLC plates using Camag linomat 5 sample applicator [16]. The plate is then developed using butanol: glacial acetic acid: water solvent system (4:1:1) up to 8 cm. After the development the plate is scanned densitometrically at 366 nm (fluorescence mode) using Camag linomat TLC scanner 3 and photo documented using TLC visualizer 2. After this the plate is derivatized using anisaldehyde sulphuric acid reagent and heated the plate at 110°C for 5 minutes. Then the plates are scanned densitometrically at 580 nm using TLC scanner3 and photo documented using TLC visualizer 2.

## 2.7 Polyphenol Profiling and Quantification Using LC-MS

“The quantitative analysis of 28 polyphenols catechol, catechin, quinine, naringenin, tocopherol, gallic acid, chlorogenic acid, epicatechin, syringic acid, vanillic acid, caffeic acid, epigallocatechin, ferulic acid, myricetin, quercetin, para-Coumaric acid, luteolin, Apigenin, kaempferol, rutin, daidzein, hesperidin, shikimic acid, ellagic acid, morin, genistein, cinnamic acid, chrysin were performed by LC-MS system (Nexera with LC-MS- 8045 Shimadzu corporation, Kyoto, Japan)- HPLC (Nexera LC-30AD) equipped with an autosampler (SIL-30AC), temperature controlled column oven (CTO-20AC) and prominence diode array detector (SPD-M20A) coupled to triple quadrupole mass spectrometer. Working standards were prepared by diluting the stock solution with water concentration ranging from 0.01 to 1µg/ml. The quantification of the polyphenols was carried out on Shimadzu Shim-Pack GISS C18 column (150* 2.1mm I.D, 1.9µm) that used 0.1% formic acid as mobile phase A and 100% methanol as mobile phase B. Polyphenols were eluted with a linear gradient system as follows. 0.5 to 1.9 min – 5% of solvent B, 2 to 10 min- 98% of solvent B, 10.1 to 15 min -98% of solvent B and 15.1 to 17 min -5% of solvent B with a flow rate 0.3ml/min, injection volume was 10µL and oven temperature $40^0$C. Positive and negative modes of multiple reaction monitoring (MRM) mode were operated during LC-MS/MS with Electron Spray ionization (ESI). LC-MS/MS data were collected and processed by LabSolutions software Shimadzu corporation, Kyoto, Japan and interface temperature of $400^0$C was conditioned for ionization, desolvation line temperature of 300°C, heat block temperature of 400°C, nebulizing gas flow (Nitrogen) at 3L/min and drying gas (Nitrogen) 10L/min. Each calibration solution was analyzed in triplicate and the average value of the result was used as the representative for each point” [17].

## 3. RESULTS

**Phytochemical screening:** Phytochemical screening of HPEE and HPAE extracts.

Phytochemical screening of *H. polyrhizus* was conducted to determine the probable phytochemicals in both the extracts. The ethanolic rind extract consists of tannins, alkaloids, flavonoids, coumarins, carbohydrates, triterpenoids, glycosides, proteins and aqueous extracts consists of tannins, alkaloids, flavonoids, coumarins, carbohydrates, triterpenoids, glycosides, proteins, saponins, phenolic compounds and terpenoids (Table 1).

## 3.1 Quantitative Determination

**Thin layer chromatography:** The aqueous extract (HPAE) and ethanol extract (HPEE) has showed eight major bands in the solvent system of (n-butanol; glacial acetic; water (4;1;1)). The Rf values of the bands of HPAE are 0.029, 0.170, 0.284, 0.427, 0.630, 0.787, and 1. 000.The Rf values of the bands obtained from the ethanol extracts are 0.013, 0.170, 0.270, 0.426, 0.515, 0.649, 0.801, and 1.000.

**High performance thin layer chromatography:** HPTLC profile of aqueous and ethanolic extract of *H. polyrhizus* has been shown in Fig. 1. The peak no.2 of aqueous extract exhibited maximum percentage area which was 40.48% (Fig. 2) with a height of 36.79 (Table 3) and minimum percentage area was present in peak no.6 with 1.76%. Therefore peak no.2 may be the chemical constituent that is present in the aqueous extract. Whereas the peak no.8 of ethanol extract exhibited the maximum percentage area which was 45.86% with a height of 34.06% (Table 4) and minimum percentage area was present in peak no.1 with an area of 0.06%.

**Table 1. Phytochemical screening of *H. polyrhizus***

| Sl. No. | Secondary metabolites | HPEE | HPAE |
|---|---|---|---|
| 1 | Tannins | +++ | ++++ |
| 2 | Alkaloids | +++ | ++++ |
| 3 | Saponins | + | +++ |
| 4 | Glycosides | ++ | +++ |
| 5 | Flavonoids | +++ | ++++ |
| 6 | Coumarins | +++ | ++++ |
| 7 | Carbohydrates | ++++ | ++++ |
| 8 | Triterpenoids | +++ | ++++ |
| 9 | Proteins | ++ | +++ |
| 10 | Phenolic compounds | + | + |
| 11 | Steroids | ++ | +++ |
| 12 | Terpenoids | ++ | +++ |

**Table 2. Quantitative phytochemical analysis**

| Samples | Alkaloids (mg/ml) | Flavonoids (mg/ml) | Tannins (mg/ml) |
|---|---|---|---|
| HPEE | 0.49±0.00 | 0.37±0.00 | 0.75±0.00 |
| HPAE | 0.58±0.10 | 0.45±0.10 | 0.92±0.10 |

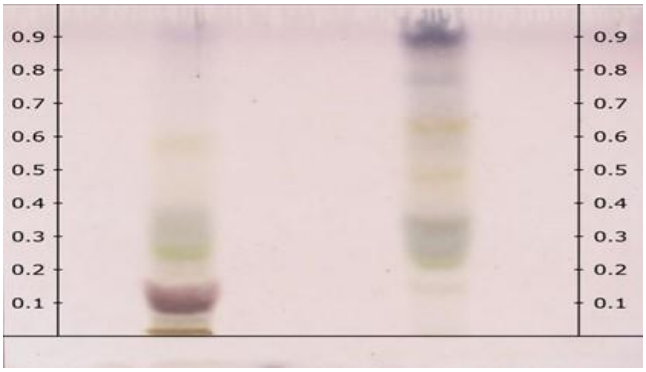


**Fig. 1. HPTLC**

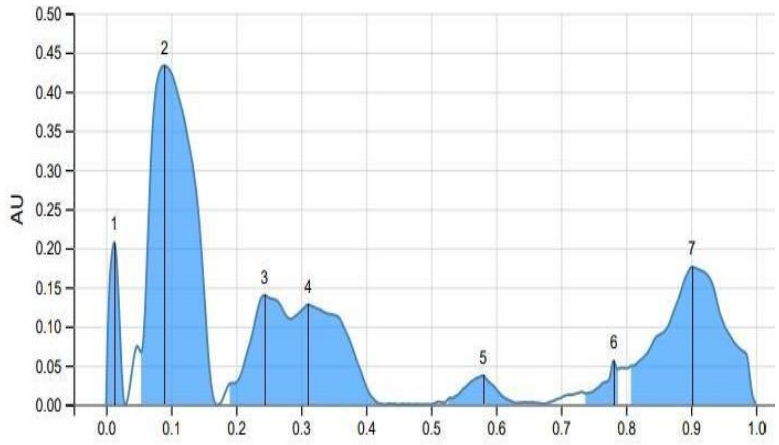


**Fig. 2. HPTLC profile of aqueous extract**

**Table 3. $R_f$ value of aqueous extract**

| PEAK | Start | | Max | | | End | | Area | |
|---|---|---|---|---|---|---|---|---|---|
| # | $R_f$ | H | $R_f$ | H | % | $R_f$ | H | A | % |
| 1 | 0.000 | 0.0000 | 0.013 | 0.2069 | 17.58 | 0.029 | 0.0000 | 0.00356 | 4.39 |
| 2 | 0.054 | 0.0668 | 0.090 | 0.4330 | 36.79 | 0.170 | 0.0000 | 0.03277 | 40.48 |
| 3 | 0.190 | 0.0266 | 0.244 | 0.1398 | 11.88 | 0.284 | 0.1093 | 0.00903 | 11.15 |
| 4 | 0.284 | 0.1093 | 0.311 | 0.1280 | 10.78 | 0.427 | 0.0000 | 0.01199 | 14.82 |
| 5 | 0.526 | 0.0071 | 0.580 | 0.0373 | 3.17 | 0.630 | 0.0020 | 0.00203 | 2.50 |
| 6 | 0.738 | 0.0140 | 0.781 | 0.0560 | 4.76 | 0.787 | 0.0452 | 0.00142 | 1.76 |
| 7 | 0.805 | 0.0478 | 0.901 | 0.1760 | 14.95 | 1.000 | 0.0000 | 0.02016 | 24.91 |

**Table 4. $R_f$ value of ethanolic extract**

| PEAK | Start | | Max | | | End | | Area | |
|---|---|---|---|---|---|---|---|---|---|
| # | $R_f$ | H | $R_f$ | H | % | $R_f$ | H | A | % |
| 1 | 0.000 | 0.0000 | 0.004 | 0.0146 | 0.81 | 0.013 | 0.0000 | 0.00009 | 0.06 |
| 2 | 0.092 | 0.0010 | 0.142 | 0.0619 | 3.45 | 0.170 | 0.0293 | 0.00252 | 1.66 |
| 3 | 0.170 | 0.0293 | 0.253 | 0.2389 | 13.31 | 0.270 | 0.2248 | 0.01585 | 10.44 |
| 4 | 0.270 | 0.2248 | 0.329 | 0.2604 | 14.51 | 0.426 | 0.0726 | 0.02798 | 18.43 |
| 5 | 0.426 | 0.0726 | 0.480 | 0.1221 | 6.80 | 0.515 | 0.1054 | 0.00892 | 5.87 |
| 6 | 0.601 | 0.1782 | 0.623 | 0.2061 | 11.48 | 0.649 | 0.1865 | 0.00928 | 6.11 |
| 7 | 0.726 | 0.1739 | 0.770 | 0.2796 | 15.58 | 0.801 | 0.2438 | 0.01757 | 11.57 |
| 8 | 0.811 | 0.2442 | 0.894 | 0.6114 | 34.06 | 1.000 | 0.0000 | 0.06965 | 45.86 |

**Table 5. Phytochemical profile**

| SI.NO. | Phytoconstituents | Methanol (Con – mg/kg) | Water (Con – mg/kg) |
|---|---|---|---|
| 1 | Catechol | 0 | 0 |
| 2 | Catechin | 2.148 | 1.761 |
| 3 | Quinine | 0.037 | 0.001 |
| 4 | Arginine | 2.391 | 2.418 |
| 5 | Tocopherol | 2.287 | 2.34 |
| 6 | Gallic acid | 0.537 | 0 |
| 7 | Chlorogenic acid | 0 | 0 |
| 8 | Epicatechin | 1.886 | 1.915 |
| 9 | Syringic acid | 0 | 0 |
| 10 | Vanillic acid | 0 | 0 |
| 11 | Caffeic acid | 0.135 | 0.13 |

| SI.NO. | Phytoconstituents | Methanol (Con – mg/kg) | Water (Con – mg/kg) |
|---|---|---|---|
| 12 | Epigallo Catechin | 1.821 | 2.732 |
| 13 | Ferulic acid | 0 | 0 |
| 14 | Myricetin | 0.927 | 0.926 |
| 15 | Quercetin | 0 | 0 |
| 16 | p- Coumaric acid | 0 | 0 |
| 17 | Luteolin | 0 | 0 |
| 18 | Apigenin | 0.53 | 0.55 |
| 19 | Kaempferol | 0.577 | 2.262 |
| 20 | Rutin | 0 | 0 |
| 21 | Daidzein | 0 | 0 |
| 22 | Hesperidin | 0 | 0 |
| 23 | Shikimic acid | 3251.645 | 912.907 |
| 24 | Ellagic acid | 0 | 0 |
| 25 | Morin | 0 | 0 |
| 26 | Genistein | 0.56 | 0.65 |
| 27 | Cinnamic acid | 0 | 0 |
| 28 | Chrysin | 0.889 | 0.873 |

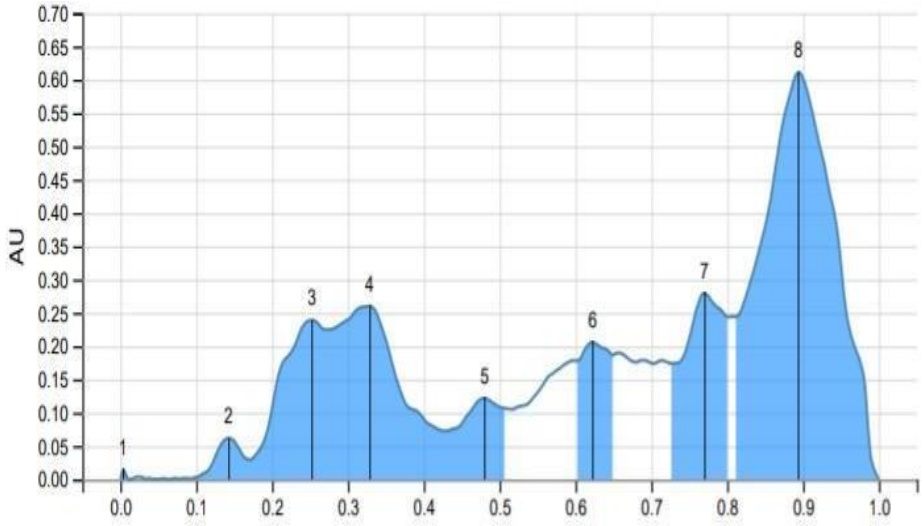


**Fig. 3. HPTLC profile of ethanolic extract**

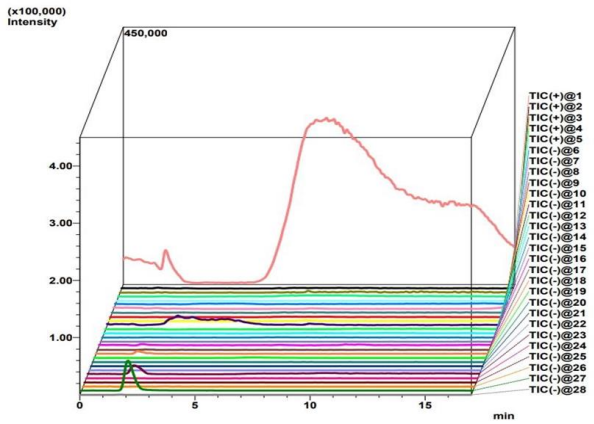


**Fig. 4. Chromatogram of aqueous extract**

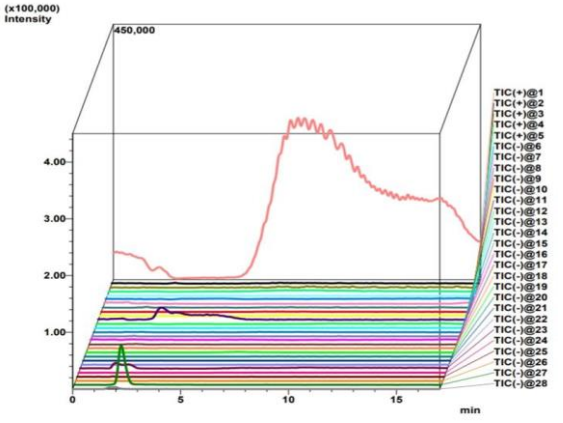


**Fig. 5. Chromatogram of ethanolic extract**

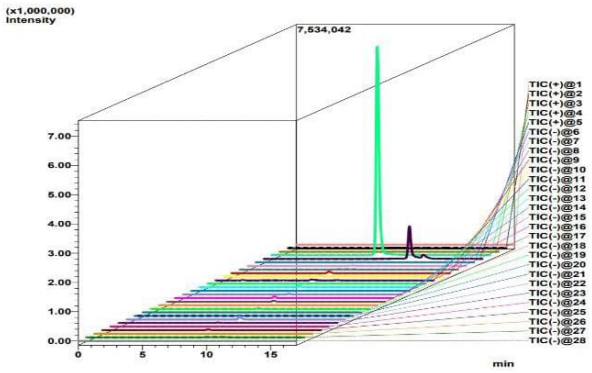


**Fig. 6. Chromatogram of standard compound**

**LC-MS analysis:** The phytochemical profiling of the aqueous and ethanolic extracts were performed using LC-MS analysis. The analysis was carried out for 28 phytoconstituents (Table 5). Major compounds that were identified belongs to various classes such as tannins, alkaloids, flavonoids, phenolic acids, vitamins and amino acids. The major bioactive compounds that are present within the aqueous extract are Epigallocatechin with concentration of 2.732mg/kg, kaempferol (2.262 mg/kg),epicatechin(1.915 mg/kg), naringenin (2.418mg/kg), tocopherol (2.34mg/kg), shikimic acid (912.907 mg/kg), myricetin (0.926mg/kg), chrysin (0.873mg/kg), genistein (0.65mg/kg), apigenin (0.55mg/ml) whereas the bioactive that are present within the ethanolic extracts were analyzed as catechins(2.148mg/ml),with major concentration and other bioactive compounds are naringenin(2.391m/ml), tocopherol (2.287mg/ml), epicatechin (1.886mg/kg), Epigallocatechin (1.821mg/ml), myricetin (927mg/ml), shikimic acid (3251.645mg/ml), gallic acid (0.537mg/ml), genistein (0.56 mg/ml), chrysene (0.889 mg/ml).

## 4. DISCUSSION

*H. polyrhizus* is a large source of nutrients as it possesses wide variety of phytoconstituents. Due to the presence of various phytoconstituents they are known to exhibit various pharmacological properties. The aqueous and ethanolic rind extracts of *H. polyrhizus* produce a large number of secondary metabolites such as tannins, alkaloids, flavonoids, phenolic acids, polyphenols, terpenoids that may serve as a reservoir for novel drugs and treatment of various ailments. Through the preliminary phytochemical screening, qualitative analysis of the rind extracts was carried out to determine the presence of metabolites. “The major phytoconstituents such as tannins, alkaloids and flavonoids were also quantitatively determined. Phytochemical profiling of the phytoconstituents present within the extracts were also carried out by HPLC. Through LC-MS, around 28 phytoconstituents were analyzed and the concentration of major constituents belonging to classes such as polyphenols, tannins, flavonoids and alkaloids were determined. Secondary metabolites like phenols have shown to have antibacterial, antineoplastic and anthelmintic activities by various researches” [18]. Flavonoids have been reported on their effective antioxidants, anticancer, antibacterial, cardioprotective, anti-inflammatory, immune system promoting [19] and tannins are known to have antioxidant, antimicrobial, antiviral, anti-mutagenic, anti-cancer, anthelmintic, hepatoprotective effects and nutritional benefits. Therefore, the presence of these secondary metabolites has been contributed to its medicinal and physiological activities [20].

## 5. CONCLUSION

The phytochemical profile of *H. polyrhizus* aqueous and ethanolic rind extracts were characterized using HPTLC and LC-MS analysis. The aqueous rind extracts, HPAE was found to contain major concentrations of Epigallocatechin, epicatechin, catechin, naringenin, myricetin, tocopherol, myricetin, kaempferol and shikimic acid belonging to various classes such as polyphenols, tannins, flavonoids, phenolic acids, amino acids and vitamins .The ethanolic extracts of *H. polyrhizus*, HPEE was found to contain high amount of catechin, naringenin, tocopherol, shikimic acid and minor concentration of myricetin, gallic acid, quinine, caffeic acid. Both

the extracts were found to have various phytoconstituents that is responsible for pharmacological properties.

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## CONSENT AND ETHICAL APPROVAL

It is not applicable.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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