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Chemical Composition Of Essential Oils From Rhazyastricta And Its Antioxidant Activity

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Abstract

Rhazyastricta is a medicinal plant. It is used for the treatment of many diseases such as Diabetes, Coating infections, cancer and inflammations. The chemical constituents of R. stricta essential oils were analyzed by using GC-FID. Seven components were analyzed from essential oils of R.stricta. These chemical components were Acetyl Pyridine (0.04%), Pyrazine (0.10%), Lavandulol (0.03%), Pinocarveol (0.02%), Hexadienolisobutanoate (0.14%), Silphiperfol-4,7 (14)-diene (0.04%) and Cubenol (1.22%). Total phenolic contents and total flavonoid contents of essential oils of R. strictawere also assessed and were found in the range of 13.5 ± 0.3 (mg GAE/g) and 3.53 ± 0.40 (mg QE/g). The antioxidant activity of essential oils of R. stricta was analyzed by using DPPH free radical scavenging assay. The inhibition % for DPPH was 23.33 ± 0.15 µg/mL which was done by essential oils of R. stricta. Chemical composition of essential oils of R. stricta was determined for the first time.

Keywords: Rhazyastricta, essential oils, GC-FID,total phenolics,total flavonoids

Introduction

Medicinal plants are used in herbal medicines and also have greater medicinal efficacy. These medicinal vegetations have constituents which are used for progress and amalgamation of drugs. These medicinal plants show key part in the expansion of human belief all over the world. Some vegetations deliberate as significance base of diet and due to this reason these vegetations suggested for their beneficial standards (Rasool Hassan, 2012). Plant kingdom has one of the greatest family which is known as Apocynaceae. Apocynaceae family comprise of genera which are 424 and species which are 4600. Plants which belongs to Apocynaceae family are inherent in Pakistan, Sri Lanka, India, China and Bangladesh (Bhadane, Patil, Maheshwari, & Patil, 2018). Rhazyastricta common name in Pakistan is “Sahwar” and it

is called “Izrushk” in balochi language in Pakistan. Its Arabic name is “Harmal” and its Persian name is “Eshvarak” (Marwat, Usman, Shah, Anwar, & Ullah, 2012). Rhazya is inherent to Asia to Southwest and it was designated in the year of 1835 as genus. Two types of persistent basils and bushes present in it. It is dispersed all over the zones which are subtropics specifically in Pakistan and India which belongs to South Asia and Arabian Peninsula which belongs to Central East (Abdul-Hameed et al., 2021). Two types of species which are known as R. orientalis and R. stricta included in this genus and this genus belongs to family Apocynaceae. R. stricta is small perennial woody shrubbery plant which is also a poisonous plant and it is also vertical and exposed (Akhgari et al., 2015). The tallness of R. stricta is 90 cm. Its branches densified, leaves are alternative and stem is smoothy. With passage of time the color of leaves changed to yellow. Its seeds are small aerial. Its fruit are light yellowish (Marwat et al., 2012). R. stricta a small goblin bush which is generally dispersed in different areas of Pakistan such as Khyber, Balochistan, NWFP, Karachi, Sindh, Dargai, grasslands which are present among Jhelum and Indus and salty series. The leaves have vertical shells (Sultana & Khalid, 2010). R. stricta has many branches which are compact and these branches climbing from foundation and also evergreen. R. stricta is basically firm, vertical climber. Its branches majorly erected from the starting point and its stem is horizontal and central(Akyalcin, Ozen, & Dulger, 2006).

Its leaves have smoothy exterior. They are 12 cm above the dwarf trunk, 10 cm extensive and 1.5 cm exteriorly. The nature of leaves is fibrous and they have alternative edge which elongated near the starting point. They also have a midrib which is protruding. They have severe top. Leaves are also oval and rigid. They are sedentary and vertical from whole edges. Its flowers length is 2.5 cm and their color are white and they are present close to spike of twigs. Flowers are wing shaped and ambisexual. They have small trunk in which stamens are injected. The color of petal is white. The length of corolla is for about 1.4 cm and 4mm for calyx. The color of corolla is also white. The lobes are intensively critical from three sided. The lobes are 15 mm in length and they have a tube which is greenish brown and this tube extended overhead the center. The lobes are basically oval and their top part is curved. Their length is greater than tray like branch. The lobes which are present in appendages are bluish from backside and they are white from inside. They are also thick headed. The stigma is annular. The filaments are small and thread like. Three types of stomata are present in R. stricta which are irregular, unequal and parallel (Bukhari, Al-Otaibi, & Ibhrahim, 2017).

Table 1. Fragments of Rhazyastricta which are used for different disorders

Disorders	**Fragment Used**
Diabetes	Entire plant
Coating infections	Ovary, Greeneries
Cancer	Greeneries
Injuries	Entire plant
Inflammations and spots problem of face	Greeneries
Helminthiasis	Entire plant

Plant R. stricta is mainly practiced for curing different kinds of illnesses in Iran, Qatar, Iraq, Afghanistan, Pakistan and India. It is practiced for medication of cancer, tumor and diabetes. Different fragments obtained form it used as antioxidant, antiviral, antifungal, antibacterial, antimutagenic, anti-inflammatory. Folk medication also practices it for treatment of pain occurring in stomach, rashes occurring on skin, sore occurring in throat and for eyes swallowing (Lanjwani, Ganghro, & Khuhawar, 2018). R. stricta examined as chief medicinal plant practice as medication in conventional drugs. And they contribute key part for curing animal and humanoid illnesses (Al-Hasawi & Al-Harbi, 2014). R. stricta actual practice is defined in outmoded medication had ascribed due to existence of alkaloids which are called as indole. When R. stricta was examined than it was identified that constituents which are alkaloid have greater biologic actions (A. I. Elkady, 2013).R. stricta which is chief medication plant show anti-cancerous and anti-oxidant qualities and also have free radical foraging belongings and used in old style medication. The research was concluded to discover the anti-cancerous efficacy of alkaloids which are obtained from R. stricta for cancer cell line which is A549. Crude alkaloid extract of Rhazyastricta remarkably improve ability of cisplatin as anti-proliferative and repress the development of cancer cell line A549(A. I. Elkady, Hussein, & Abu-Zinadah, 2014). Distillate which obtained from R. stricta used for curing different disorders such as rheumatism, helminthiasis and diabetes. Further, it was described that crude distillate gained from R. stricta cause inhibition of propagation of cells and persuade the death of cells of apoptosis in cancerous lines such as MB-231 and MCF-7. More than 100 different kinds of alkaloids are separated, categorized and recognized form the leaves, stems and roots of R. stricta. The reality is that alkaloids which isolated from R. stricta are chief significant phytochemicals which are recognized due to anti-metastatic and anti-proliferation potentials on several kinds of cancerous cells in-vivo and in-vitro respectively(Lu, Bao, Chen, Huang, & Wang, 2012). The previous work on R. stricta is available on plant extraction but the current research work is done for extraction of EOs from R. stricta.The main objective to conduct present research was to extract EOs from R. stricta, to characterize EOs by using GC-FID and to check antioxidant activity of EOs.

Materials and Methods

Collection and identification of plant material

The plant sample was collected from hilly areas of District D.G Khan, Punjab, Pakistan. The plant sample was collected from this area in the month of March. The plant sample was identified by botanist(Dr. Mansoor Hameed), University of Agriculture, Faisalabad. The research work was done at Organometallic and Coordination Chemistry Laboratory of Postgraduate Agriculture Research Station (PARS), University of Agriculture Faisalabad.

Chemical and reagents

n-hexane (DAEJUNG, Korea), Distilled water and Celite (DAEJUNG, Korea) were used for extraction of essential oils. And for biological activities Folin-Ciocalteu (FC) reagent, Sodium carbonate(SIGMA-ALDRICH, Germany), Sodium nitrate(SIGMA-ALDRICH, Germany), Aluminum chloride(SIGMA-ALDRICH, Germany)and DPPH solution were used.

Extraction of essential oils

Hydro-distillation method was basically used for extraction of essential oils from R. stricta. First of all, collection of plant sample was done. The plant sample was washed with the help of distilled water. After it plant sample was dried for some time to evaporate water. The plant sample was raptured slightly with help of pestle and mortar and was weighted with the help of analytical balance. Total plant sample was for about 8560g. Distillery was ON and water supply was also started in distillery and after sometime distillery was filled up with water. A flask of about 500 mL was linked with distillery for collection of water and EOs. First batch of for about 2140g was put in distillery containing water and its led was covered. After sometime distillery was boiled up and finally boiling of distillery water along with EOs passing through pipe which linked with distillery and one end of pipe is in the flask in which water and EOs start collected. Drop by drop water and EOs start collected. The upper layer was EOs layer and lower layer was water layer. When flask filled up with water and EOs lower water layer was removed from flask upper layer of EOs remains as it was earlier. This process continues for about 2 hours than after it first batch of plant sample was removed and second batch of plant sample was added in the same way. Same procedure was repeated for third and fourth batch of plant sample. This procedure was continued for about 8 to 9 hours. Lower water layer was removed and upper EOs layer remains as it was before and become thick was passage of time. When whole procedure was completed than water was completely removed from flask than some quantity of n-hexane was added in flask containing EOs. So EOs which present on walls of flask moved in n-hexane. Finally, n-hexane containing EOs was passed through five-layer filter paper which is present in funnel filled with celite for removal of water so that pure EOs obtained. After sometime whole sample passed through 5-layered filter paper and pure EOs was obtained. The pure EOs was put in 5mL glass vails(Elyemni et al., 2019).

Characterization of essential oils

GC majorly used for volatile constituent's analysis. And an FID is systematic apparatus that calculate analytical sample in gaseous streamlet. Detector FID commonly used in GC. The method through which concluding information exhibited and it mainly relays on software and computer. Mostly FID is linked to GC arrangement. The EOs GC-FID examination was achieved with GC (Perkin Elmer) with Clarus (480) which is fortified with detector such as FID and a tube column like Elite-5 (PerkinElmer; 35 m × 0.30 mm × 0.30 µm). The temperature of oven was automated. The temperature of injector and detector were 220°C and 280°C, correspondingly. The (99.99%) helium at 0.6 (mL min^{-1}) drift rate was used as carrier gas. The EOs sample of for about 2 µL were inoculated with help of split mode. The EOs % age composition was determined by means of retention timeand peak area(Silva-Flores et al., 2019).

Antioxidant activity

Total phenolic contents (TPC)

2µLFolin-Ciocalteu (FC) reagent was taken in culture tube. Then 800µL of Na_2CO_3 was added in culture tube containing FC reagent. After it 1mL of essential oil sample was added in culture tube containing FC reagent and Na_2CO_3. This culture tube was left for two hours. After two hours small amount was taken from this culture tube and added on ELISA plate. This ELISA plate was placed in spectrophotometer and reading was taken on 765nm wavelength. The calculation of total phenolic contents was done with help

of gallic acid calibration curve. The outcomes were represented as gallic acid equivalence (GAE) per dry weight. The experiment was repeated in a triplicate way (Anwar, Ali, Hussain, & Shahid, 2009).

Total flavonoid contents (TFC)

1.25mL distilled water was taken in culture tube. Then 250µL essential oil sample was added culture tube containing distilled water. Then 75µL of $NaNO_2$ was added in culture tube which contain distilled water and essential oil sample. After it 150µL of $AlCl_3$ was added in culture tube which already contain distilled water, essential oil sample and $NaNO_2$. It was left for five minutes. After five minutes 250µL amount from culture tube was taken and added on ELISA plate. Then this ELISA plate was placed in spectrophotometer and reading was taken on 510nm wavelength. Three readings were taken at 510 nm wavelength. The total flavonoid contents were represented as quercetin equivalent (QE) per gram of dry weight. The experiment was repeated three times(Riaz et al., 2012).

DPPH radical scavenging assay

Firstly, 1mL of DPPH solution was taken in Eppendorf tube. Then 10µL of essential oil sample was added in Eppendorf tube containing DPPH solution. Eppendorf tube containing DPPH solution and sample was left for half hour in dark place because it was light sensitive. After half hour Eppendorf tube containing DPPH solution and sample was taken. 250µLamount from Eppendorf tube was taken and added on ELISA plate. Then ELISA plate was placed in spectrophotometer and reading was taken on 510nm wavelength. Three readings of absorbance of blank and sample solution were taken at 510nm wavelength. The % inhibition of DPPH was calculated by this formula.

DPPH inhibition (A. I. J. G. Elkady & biology) = $100 \times (A_{blank} - A_{sample} / A_{blank})$

A_{blank} is the absorbance of control reaction mixture without including the test ofblank solution compounds and $A_{sample}$is the absorbance of sample solution(Abdullah I Hussain, Anwar, Shahid, Ashraf, & Przybylski, 2010).

Results and discussion

Chemical composition of EOs of R. stricta

The first peak at retention time 1.96 (minute) is the peak of solvent (n-Hexane, 98.41%) which is used for the extraction of EOs from R. stricta. The second peak with retention time 8.84 (minute) is the peak of Acetyl Pyridine. Third peak with retention time 11.23 (minute) is the peak of Pyrazine. Forth peak with retention time 13.95 (minute) is the peak of Lavandulol. Fifth peak with retention time 15.00 (minute) is the peak of Pinocarveol. Sixth peak with retention time 15.92 (minute) is the peak of Hexadienoliso but a noate. Seventh peak with retention time 22.77 (minute) is the peak of Silphiperfol-4,7 (14)-diene. Last eighth peak with retention time 34.65 (minute) is the peak of Cubenol. So identified compounds by GC-FID are Acetyl Pyridine (0.04%), Pyrazine (0.10%), Lavandulol (0.03%), Pinocarveol (0.02%), Hexadienoliso but a noate (0.14%), Silphiperfol-4,7 (14)-diene (0.04%) and Cubenol (1.22%) which are presented in (Table 2)and the structures of all these identified compounds are shown in (Figure 2)(Adams, 2007). The chromatogram of EOs of R. stricta is shown in (Figure 1).

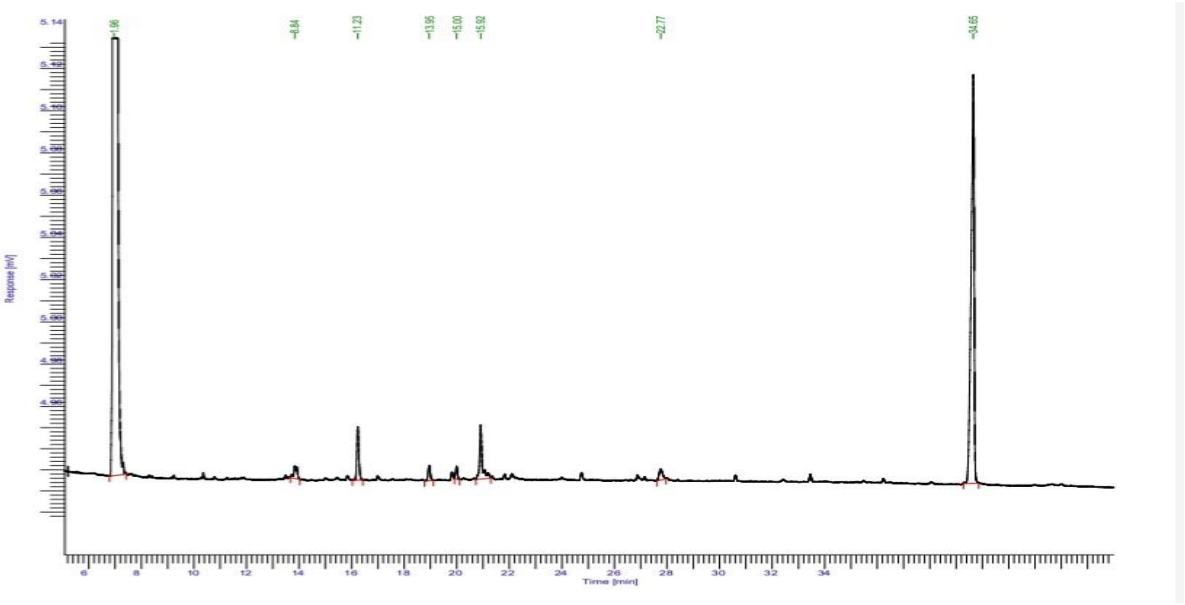
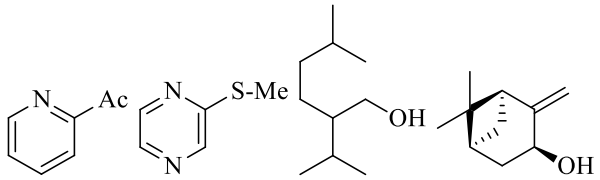


Figure 1. GC-FID chromatogram of R. stricta EOs

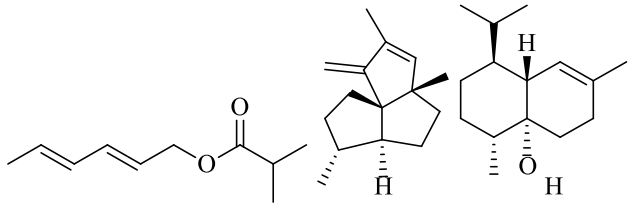
Table 2. R. stricta EOs identified compounds by GC-FID

Peak No	Retention Time	Area	Compound	Formula
1	1.96	98.41	n-Hexane (Solvent)	C_6H_{12}
2	8.84	0.04	Acetyl Pyridine	C_7H_7NO
3	11.23	0.10	Pyrazine	$C_5H_6N_2S$
4	13.95	0.03	Lavandulol	$C_{10}H_{22}O$

5	15.00	0.02	Pinocarveol	$C_{10}H_{16}0$
6	15.92	0.14	Hexadienoliso but a noate	$C_{10}H_{16}0_2$
7	22.77	0.04	Silphiperfol-4,7 (14)-diene	$C_{15}H_{22}$
8	34.65	1.22	Cubenol	$C_{15}H_{26}O$



Acetyl Pyridine Pyrazine Lavandulol Pinocarveol



HexadienolisobutanoateSilphiperfol-4,7 (14)-diene Cubenol

Figure 2. Structures of compounds identified by GC-FID

Total phenolic and total flavonoid contents

The total phenolic contents and total flavonoid contents were 13.5 ± 0.3 (mg GAE/g) of dry matter and 3.53 ± 0.40(mg QE/g) of dry matter. Total phenolic contents were determined by Folin-Ciocalteu (FC) process. Gallic acid was taken as a standard for determination of total phenolic contents. Quercetin was taken as a standard for determination of total flavonoid contents. The total phenolic contents were greater as compared to total flavonoid contents in the EOs of R. stricta. The total phenolic contents have redox properties which are accountable for activity as antioxidant(Tohidi, Rahimmalek, & Arzani, 2017). The total phenolic and flavonoid contents of EOs of R. stricta are shown in (Table 3) and total phenolic and flavonoid contents are shownin (Figure 3).

Table 3. Total phenolic contents and Total flavonoid contents of R. stricta EOs

Rhazyastricta EOs	**TPC (mg GAE/g) of dry matter**	**TFC(mg QE/g) of dry matter**
	13.5 ± 0.3	3.53 ± 0.40

The results are represented as mean ± SD of triplicate experiments

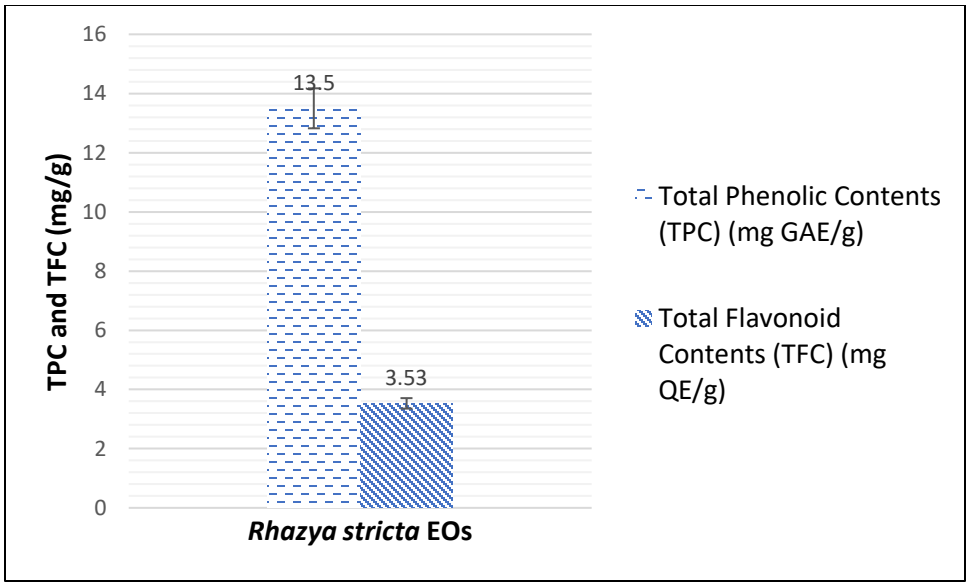


Figure 3. Total phenolic contents and total flavonoid contents of EOs

ofR. stricta

DPPH radical scavenging assay

DPPH standard solution was used to determine antioxidant activity of EOs of R. stricta. DPPH solution has purple color. The EOs of R. stricta changed the purple color of DPPH into yellow color. The stable color of DPPH was purple. Due to receiving of proton from EOs the purple color of DPPH changed into yellow color. The inhibition % was 23.33 ± 0.15 µg/mL which was done by essential oils of Rhazyastrictafor DPPH free radicalscavenging activity (Abdullah Ijaz Hussain, Anwar, Sherazi, & Przybylski, 2008). Antioxidant activity of EOs of R. stricta for DPPH assay is shown in (Table 4) and DPPH inhibition done by EOs of R. stricta is shown by bar graph in (Figure 4).

Table4. Antioxidant activity of EOs of R. stricta for DPPH assay

Rhazyastricta EOs	**DPPH inhibition µg/mL**
	23.33 ± 0.15

The result is represented as mean ± SD of triplicate experiments

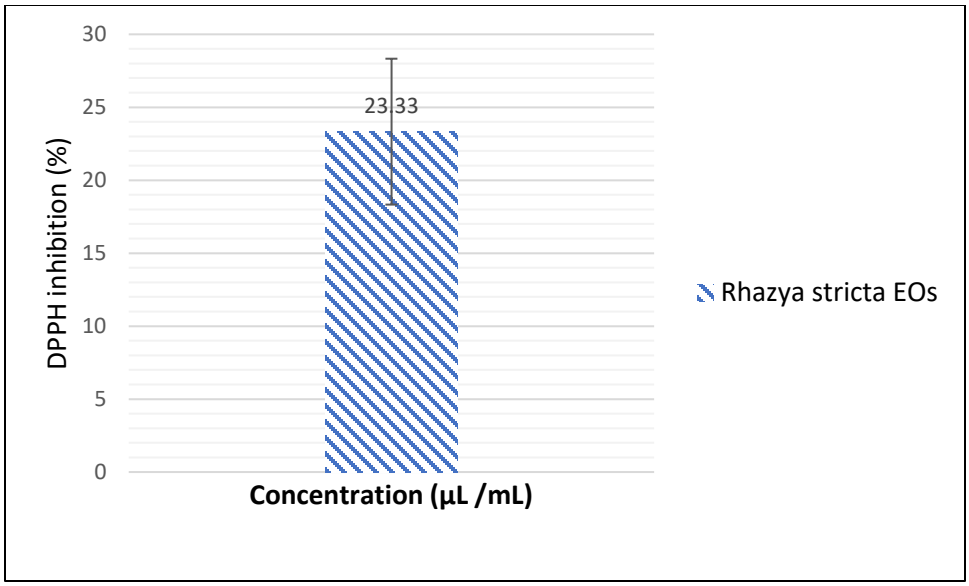


Figure 4. DPPH inhibition done by R. strictaEOs

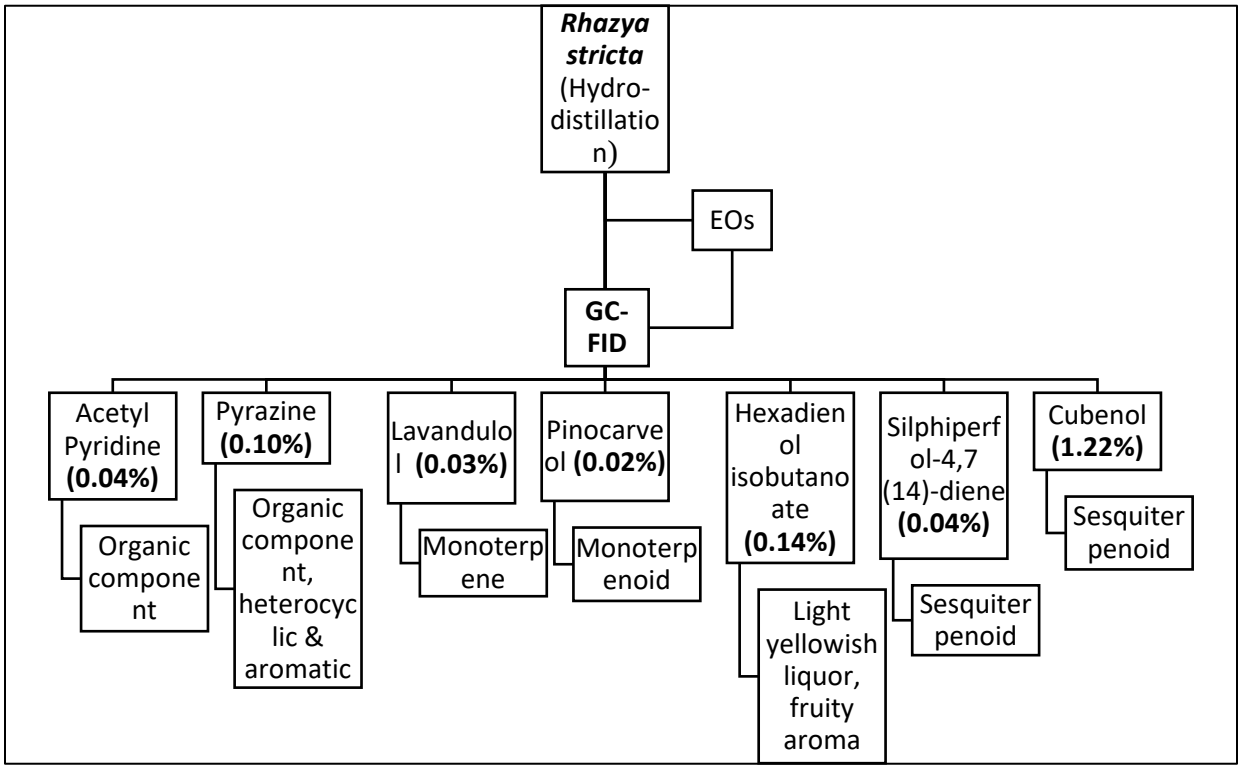


Figure 5. Schematic representation of characterization of R. stricta EOs by GC-FID

Schematic representation of characterization of EOs of R. stricta by GC-FID is shown in (Figure 5). The first compound which is identified by GC-FID is Acetyl Pyridine which is organic component. The second compound is Pyrazine which is also organic component. Third compound is Lavandulol which is monoterpene. Forth compound is Pinocarveol which is monoterpenoid. Fifth compound is Hexadienoliso

but an oate which is light yellowish liquor with fruity aroma. Sixth compound is Silphiperfol-4,7 (14)-diene which is sesquiterpenoid. The last compound is Cubenol which is also sesquiterpenoid(Adams, 2007).

Conclusion

The chemical composition of essential oils of R. stricta is evaluated for first time. The chemical composition examination was done by using GC-FID technique. Seven components were identified by GC-FID. These components are Acetyl Pyridine (0.04%), Pyrazine (0.10%), Lavandulol (0.03%), Pinocarveol (0.02%), Hexadienoliso but a noate (0.14%), Silphiperfol-4,7 (14)-diene (0.04%) and Cubenol (1.22%). Total phenolic and flavonoid compounds which obtained from R. stricta were 13.5 ± 0.3 (mg GAE/g) and 3.53 ± 0.40 (mg QE/g). The % inhibition which was shown by EOs of R. stricta was 23.33 ± 0.15 µg/mL. In future characterization of EOs of R. strictacan be done by using Raman spectroscopy. And activity of EOs of R. strictasuch as anticancer and antimicrobial can also be checked.

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CONFLICTS OF INTEREST

The authors have no conflicts of interest.

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