**GC-MS Analysis of Bioactive Compounds Extracted from Plant *Rhazya* *stricta* Using Various Solvents**

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 **Abstract**

The effects of employing solvents with varying polarity on the yields of phytochemical components extracted from the plant *Rhazya stricta* have been studied. Chloroform-methanol (1:1), methanol, ethanol, diethyl ether, and ethyl acetate were utilized as extraction solvents. Results showed the efficiencies of the solvents in the extraction of phytochemical compounds were in this order: chloroform-methanol extract < ethanol extract < methanol extract < diethyl ether extract < ethyl acetate extract. Chloroform-methanol produced the highest concentration of phenolic and flavonoid contents among the five solvents tested (13.3 mg GAE/g DM and 5.43 CE/g DM). The phytochemical compounds of yield extract ranged from 47.55 to 6.05%. The results reveal that the extraction solvent properties significantly impacted the extraction yield and phytochemical components of *Rhazya stricta* extract. Furthermore, compared to the other solvents, the chloroform-methanol extraction led to the highest yield (47.55%) and greater phytochemical substances.

**Keywords**: phytochemical compounds; different solvents; *Rhazya* *stricta*

**Abbreviations:** GAE: gallic acid equivalent, DM: dry matter, CE: catechin

**1. Introduction**

Natural medicine has been used to treat diseases for many decades, but the biologically active molecules, plant-derived and mechanism of action, have been debated for years. Folkloric herbal remedies are commonly employed as a source of innovative medications in folk medicine. It has been used for decades to treat various human and animal diseases ([Hassannia et al., 2020](#_30j0zll); [Silva et al., 2019](#_1fob9te)). Varieties of therapeutic plant species are still being identified in environmentally varied places, such as the Saudi peninsula. Under adverse weather conditions, a large number of these plants grow, making their genomes remarkably unique and used in the treatment of various conditions ([Awadh Ali et al., 2017](#_2et92p0); [Ebrahim et al., 2020](#_tyjcwt); [El-Saber Batiha et al., 2020](#_3dy6vkm)). *Rhazya* *stricta* is a classic shrub that is toxic, low, erect, and glabrous. It is one of the most common medicinal shrubs in the desert of the Arab Peninsula, including Saudi Arabia, and is used in herbal medicines to treat various diseases ([Redwan et al., 2016](#_1t3h5sf)). Recently, its extracted materials have been used in the formulation of silver nanoparticles, which has a role in fighting mosquito vectors and multiple pathogens ([Aziz et al., 2020](#_4d34og8)). The *R. stricta* contains glycosides, alkaloids, tannins, and triterpenes ([Baeshen NA, 2009](#_2s8eyo1)) and is considered a rich source of indole alkaloids ([Ahmed et al., 2018](#_17dp8vu); [Akhgari et al., 2019](#_3rdcrjn)). Indole alkaloid compounds generally exhibit antinociceptive, antitumor, anti-inflammatory, antioxidant, and antimicrobial antihypertensive properties ([Rosales et al., 2020](#_35nkun2); [Yu et al., 2018](#_1ksv4uv)). More than 100 alkaloids have been identified by the phytochemical analysis methods from *R. stricta* ([Yaghmoor et al., 2015](#_44sinio)). Based on the previously mentioned facts, the current study aimed to investigate the phytochemical compounds extracted from *R. stricta* by different solvents (methanol: chloroform 1:1, diethyl ester, methanol, ethanol, and ethyl acetate). Using multiple solvents for extraction from *R. stricta* will open the window for discovering various bioactive compounds with therapeutic potential.

**2. Materials and Methods**

**2.1. Collection of Plant Samples and Preparation**

*R. stricta* plant materials were collected from the Ghola area at Osfan with coordinates N: 21.935.1966 and E: 39.305869. The collected samples were brought to the laboratory, and leaves were separated from stems, washed with running tap water, and left to dry in the shade at the laboratory for three days. When the leaves were completely dehydrated, they were placed in a blender, ground to a fine powder; and kept at room temperature for further use.

**2.2. Sample Extraction**

100 g of fine powder was extracted using 500 mL of absolute ethanol, methanol, diethyl ether, chloroform/methanol mixture (1:1, v/v), or ethyl acetate. All samples were ultrasonicated in a water bath at 40 °C for three hours, soaked in a shaking water bath at 70 °C for 24 hours until the solvent became colorless, filtered through Whatman filter paper NO.2, and analysed by GC-MS.

**2.3. Total Phenolic Content**

The method explained by Velioglu et al.,1998; was used to determine the total phenolic content. Firstly,100 μL Folin-Ciocalteu reagent was introduced to 100 μL of the plant extract and 800 μL distilled water and left for 5 min at room temperature. The reaction mixture was then given 500 μL of sodium carbonate (15%, w/v). The absorbance was measured at 750 nm after 30 min. The results were represented in mg gallic acid equivalent per gram of dry matter (mg GAE/g DM).

**2.4. Total Flavonoid Content**

The method described by Zhishen et al., 1999; was used to determine the flavonoid content. Firstly, 250 μL of plant extract, 1.25 μL distilled water, and 75 μL NaNO2 solution (5%, w/v) were combined in a reaction mixture and allowed to stand for 6 min. Then 150 μL of AlCl3 solution (10%, w/v), 0.5 mL of 1M NaOH, and 275 μL of distilled water were added to the reaction mixture and allowed to stand for 5 min. The absorbance was recorded at 510 nm. The results were calculated as mg catechin equivalent/g dry matter (mg CE /g DM). A solution of catechin was used as the standard.

**2.5. Gas Chromatography-Mass Spectrometry (GC-MS) Analysis**

The chemical compositions of samples were determined using a Thermo Scientific Trace GC1310-ISQ mass spectrometer with a direct capillary column TG–5MS (30 m x 0.25 mm x 0.25 m film thickness). Initially, the column oven was maintained at 50°C; then it was increased by 5°C/min to 230°C, which was held for 2 minutes, and then by 30°C/min to 290°C, also maintained for 2 min. The temperatures of the injector and MS transfer lines were held at 250 and 260 °C, respectively; helium was utilized as a carrier gas at a constant flow rate of 1 mL/min. The solvent delay was 3 min and diluted samples of 1 μL were injected automatically using Autosampler AS1300 coupled with GC in the split mode. EI mass spectra were collected at 70 eV ionization voltages over the range of m/z 40–1000 in full scan mode. The ion source temperature was set at 200 °C. The components were identified by comparing the components' retention times and mass spectra to those of the WILEY 09 and NIST 11 mass spectral databases.

**Results and Discussion**

* 1. **Phenolic and Flavonoids Contents**

The potential for antioxidant activity in plants is proportional to the amount of cell-reinforcing chemicals present, such as phenolic compounds that are capable of catalyzing free radical scavenging (Almulaiky et al., 2020). To extract phenolic and flavonoid chemicals, the appropriate solvent must be utilized. The capacity of several solvents to extract phenolic and flavonoid compounds from *R. stricta* is shown in Table 1. Methanol, ethanol, ethyl acetate, diethyl ether, and chloroform-methanol (1:1) were tested to determine the best solvent to extract phenolic and flavonoids. Chloroform-methanol produced the highest concentration of higher phenolic compounds among the five solvents tested (13.3 mg GAE/g DM). As well as producing a greater concentration of flavonoid content (5.43 CE/g DM). Chloroform-methanol was the best solvent for extracting polyphenolic chemicals from plants due to its ability to inhibit polyphenol oxidase activity. This enzyme causes polyphenols' oxidation and its dispersion efficiency (Yao et al., 2004). In *Caesalpinia decapetala* (Pawar et al., 2010), *Portulacaceae* (Almulaiky et al., 2020), and *Morus nigra* and *Artocarpus heterophyllus* Leaves (Thakur et al., 2020), methanol (70%) extracts were used to investigate antioxidant properties and flavonoids components.

* 1. **Extraction by Ethanol Solvent and Identification of Compounds by GC/MS**

Nineteen compounds found in *R. stricta* extract by ethanol solvent are shown in Table 2. The peak area percentage was used to indicate the relative concentration of each compound. The main compounds identified based on relative contents were 17-Octadecenoic acid, methyl ester (46.32%); Hexadecanoic acid, methyl ester (24.22%); Aspidospermidine, 1,2-didehydro-, (5à,12á,19à)- (11.34%); and Akuammilan-17-oic acid, methyl ester (3.44%). Most of the compounds extracted by ethanolare unsaturated fatty acids. Hexadecanoic acid has been detected to have a significant role in the modulation of anti-inflammatory responses in macrophages ([Korbecki and Bajdak-Rusinek, 2019](#_z337ya)). Also, it affects human semen quality ([Esmaeili et al., 2015](#_3j2qqm3)). Aspidospermidine is a bioactive alkaloid extracted from many plants and has been used as a target for synthesis ([Xu et al., 2019](#_2xcytpi)). Finally, akuammilan-17-oic acid was detected to have a promising significant antibacterial potential against *Acinetobacter baumannii* ([Skariyachan et al., 2019](#_1ci93xb)). The results come in accordance with the previous reports which prove the fatty acid profile of *R. stricta* ([Akhgari et al., 2019](#_3rdcrjn)). These results suggested the positive biological effect of bioactive materials extracted from *R. stricta* by ethanol solvent. In the same line, the high contents of fatty acid extracted from *R. stricta* demonstrate its volatile flavors which have been previously detected ([Goff and Klee, 2006](#_3whwml4)).

* 1. **Extraction by Methanol Solvent and Identification of Compounds by GC/MS**

Table 3 presents twenty-five compounds extracted by methanol solvent in Plant *R. stricta*. The main compounds identified based on relative contents were Aspidospermidine, 1,2-didehydro-, (5à,12á,19à) (28.37%); Aspidospermidine-3-carboxylic acid, methyl ester, (2á,3à,5à,12á,19à) (14.27%); Quebrachamine (11.96%); and Pyridine, 3-ethyl-(5.63%). Most of the compounds extracted by methanol are alkaloids. Along the same line, previous data proved the existence of alkaloids in *R. stricta* ([Ahmed et al., 2018](#_17dp8vu); [Bukhari et al., 2017](#_3as4poj)). Also, it has been detected that genetic diversity can affect the plant content of alkaloids ([Abd-Elgawad and Alotaibi, 2019](#_1pxezwc)). It is well-known that alkaloids are a rich source for drug discovery and formulation ([Mondal et al., 2019](#_49x2ik5)). Various alkaloids have been examined for their anticancer and antiproliferative activities ([Wada and Yamashita, 2019](#_2p2csry)). Another record has elucidated their role in providing protection to animals subjected to UV radiation ([Takshak and Agrawal, 2019](#_147n2zr)). The results obtained in the present study emphasized the potential therapeutic use of *R. stricta*. Especially as a potent source of alkaloids, with promising chances for discovering multiple bioactive materials with therapeutic properties against different malignancies.

* 1. **Extraction by Diethyl Ether Solvent and Identification of Compounds by GC/MS**

Table 4 shows the twenty-four compounds found in the Plant *R. stricta* extract by Diethyl ether solvent. The main compounds identified based on relative contents were Aspidospermidine, 1,2-didehydro-, (5à,12á,19à)- (26.76%); Squalene (22.49%); Phthalic acid, di (2-propylpentyl) ester (9.19%); and Quebrachamine (5.49%). Most of the extracted compounds by Diethyl ether are alkaloids and triterpene. Triterpenes have been proven to be existed in *R. stricta* through cheminformatics studies to determine the bioactive molecules of therapeutic potentials ([Obaid et al., 2017](#_3o7alnk)). The medicinal use of triterpenes has been elucidated for their antitumor activities ([Wang et al., 2017](#_23ckvvd)), inhibitory effect on nitric oxide (NO) production ([Fu et al., 2018](#_ihv636)), anti-inflammatory activities ([Shi et al., 2017](#_32hioqz)) and antineoplastic activities ([Pettit et al., 2018](#_1hmsyys)). Although the high therapeutic potentiality of *R. stricta*, its phthalic acid content opens discussions about the adverse effect of this bioactive compound reported in previous studies ([Chuang et al., 2020](#_41mghml); [Qiu et al., 2020](#_2grqrue)).

On the other hand, there was a high amount of squalene detected in *R. stricta*. Squalene is a polyunsaturated hydrocarbon with multiple bioactivities, including skin hydrating, emollient agent, drug carrier, antioxidant, and detoxification ([Kim and Karadeniz, 2012](#_1v1yuxt)). Recently, the great role of squalene as an adjuvant for influenza vaccines has been detected ([Beyer et al., 2020](#_4f1mdlm)), and its role in the treatment of cardiovascular disease has been determined through its statin-like action ([Ibrahim et al., 2020](#_2u6wntf)). Quebrachamine, another indole alkaloid extracted from *R. stricta,* has been detected to have blocking activities against adrenergic nerves of urogenital tissue ([Deutsch et al., 1994](#_19c6y18)). Our results are, in accordance with previous reports, detecting Quebrachamine in *R. stricta* ([Akhgari et al., 2019](#_3rdcrjn)). The extracted bioactive materials from *R. stricta* by diethyl ester tended to have significant therapeutic activity; the same prospect was reported by ([Sultana and Khalid, 2010](#_3tbugp1)). All previously mentioned records emphasize the therapeutic potential of *R. stricta* regarding its isolated and extracted bioactive compounds.

* 1. **Extraction by Chloroform-Methanol Solvent and Identification of Compounds by GC/MS**

The Sixteen compounds found in the Plant *R. stricta* extract by chloroform-methanol solvent are recorded in Table 5. The compounds identified based on relative contents were Methyl stearate (47.55%); Methyl tetradecanoate (6.03%); Aspidospermidine, 1,2-didehydro-, (5à,12á,19à)- (1.53%); and Dodecanoic acid, methyl ester (1.46%). Most of the compounds extracted by chloroform-methanol are fatty acids and alkaloids; the results of this study can be compared with previous records, which reported more than 100 alkaloid compounds extracted from *R. stricta* ([Baeshen and Khan, 2015](#_28h4qwu)). Methyl stearate, the most extracted fatty acid from *R. stricta* by chloroform-methanol, was detected to have a regulatory effect on the calcium-activated chloride channels, the matter which opened the debate for its use in drug synthesis and fabrications ([De Jesus-Perez et al., 2018](#_nmf14n)). It was also reported to have anti-inflammatory activity through its ability to down-regulate the pro-inflammatory response ([Dey et al., 2016](#_37m2jsg)). Moreover, Methyl stearate has several uses in biological and medical research ([Dey et al., 2015](#_1mrcu09)). Another bioactive compound, methyl tetradecanoate, a fatty acid extracted from *R. stricta,* has contraceptive activities ([Simbala et al., 2017](#_111kx3o)). The previously mentioned citations confirm the potentiality of *R. stricta* extracted bioactive compounds to be a potent therapeutic compound.

* 1. **Extraction by Ethyl Acetate Solvent and Identification of Compounds by GC/MS**

The forty-four compounds found by ethyl acetate solvent in the Plant *R. stricta* extract are shown in Table 6. The main compounds identified based on relative contents were Aspidospermidine, 1,2-didehydro-, (5à,12á,19à)- (6.05%); Pyridine, 3-ethyl-(4.01%); Oleic Acid (2.16%); and Vitamin E (1.94%). The *R. stricta* extract by ethyl acetate solvent resulted in higher oleic acid content. The results could be compared with previous results that reported the existence of oleic acid in *R. stricta* ([Hanif et al., 2011](#_3l18frh)). As an omega-9 unsaturated fatty acid, oleic acid has been detected to regulate female fertility, as it is involved in female germ cell growth and development. It contributes to oocyte pre-implementation and embryo growth ([Fayezi et al., 2018](#_206ipza)). Moreover, it has a beneficial role in diminishing the incidence of cardiovascular disorders, carcinogenesis, liver dysfunctions and intestinal inflammations ([Piccinin et al., 2019](#_4k668n3)). Also, it has a potent ability to mitigate the inflammatory responses in sepsis ([Medeiros-de-Moraes et al., 2018](#_2zbgiuw)), it has antioxidant power ([Guzman et al., 2016](#_1egqt2p)), anti-parasitic action against *Acanthamoeba Castellanii* Trophozoites ([Wu et al., 2018](#_3ygebqi)), it promotes the differentiation of neural cells in the human endometrial stem cells ([Kojour et al., 2017](#_2dlolyb)). Recently, oleic acid has been detected to ameliorate hepatocellular lipotoxicity ([Zeng et al., 2020](#_sqyw64)), acts as a carrier for anti-cancer drugs ([Eh Suk et al., 2020](#_3cqmetx)), up-regulates myosin heavy chain-1 expression, and elevates the mitochondrial mass in myoblasts ([Watanabe et al., 2020](#_1rvwp1q)). The high oleic acid content makes *R. stricta* a possible medicinal plant for many diseases. Vitamin E was also extracted from *R. stricta*; the same results were obtained ([Iqbal et al., 2006](#_4bvk7pj)).The biological activities and importance of vitamin E are well-known. Its antioxidant power is recently and extensively recognized in thousands of literatures ([Kemnic and Coleman, 2020](#_1664s55)). Recently, it was recognized that its low serum levels are associated with high pain and disability in osteoarthritis patients ([Eftekharsadat et al., 2020](#_3q5sasy)). Moreover, its administration after surgical operations enhances the Osseointegration of stainless-steel implants in rats ([Savvidis et al., 2020](#_25b2l0r)). The obtained results proved that *R. stricta* is a potent source of vitamin E, revealing its ability to be a potent source of antioxidants.

* 1. **Comparison between Extraction Percentage of the Phytochemical Compounds by Different Solvents**

Table 7 shows different extracts obtained using various solvents. The results indicate that methanol-chloroform extraction led to the highest yields compared to the other solvents, namely ethanol, methanol, Diethyl ether, and ethyl acetate. The proportion of chloroform-methanol extraction based on the dry weight of the extract was 47.55 %. In contrast, the extract percentages of ethanol, methanol, Diethyl ether, and ethyl acetate were 46.32 %, 28.37 %, 26.76 %, and 6.05 % (dry weight), respectively. The discrepancies in yield extracted using different solvents were caused by variances in the polarity of various plant chemicals, as described by Jayaprakasha ([Jayaprakasha et al., 2001](#_kgcv8k)). As a result of this variation, the solubility of the solvent employed differed, and the percentage of yield extracted varied depending on the kind of solvent used, implying that the high-polarity solvent would extract the most polar compounds ([Kosar et al., 2007](#_34g0dwd); [Sultana et al., 2009](#_1jlao46)).

**4. Conclusion**

This analysis investigated the effects on the phytochemical compounds derived by *R. stricta* by using solvents with different polarities. The solvents used included chloroform-methanol, ethanol, methanol, Diethyl ether and ethyl acetate. The results revealed that chloroform-methanol registered a high extraction yield and phytochemical compounds. Therefore, it can be inferred that the properties of the extraction solvents play a significant role in evaluating the effectiveness of solvents in phytochemical compounds. On the other way, the extracted bioactive compounds revealed the medicinal potentials of *R. stricta* for female reproduction disorders, cardiovascular disease, obesity, inflammatory conditions, and hepatic disorders. Moreover, it can be considered a rich source of antioxidants, alkaloids, and beneficial unsaturated fatty acids.

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Table 1 Total phenolics and flavonoids of *Rhazya stricta* extracted by different solvents. Values are the means of three replicate ± SD.

|  |  |  |
| --- | --- | --- |
| Solvent | Phenolic content (mg) | Flavonoid content (mg) |
| Chloroform-methanol  | 13.3 ± 0.86 | 5.43 ± 0.89 |
| Methanol | 6.4 ± 0.24 | 2.75 ± 0.43 |
| Diethyl ether  | 2.5 ± 0.16 | 1.12 ± 0.52 |
| Ethyl acetate | 1.61 ± 0.09 | 0.63 ± 0.39 |
| Ethanol | 8.32 ± 0.45 | 3.87 ± 0.21 |

Table 2. Phytochemical compounds of *Rhazya* *stricta* extracted by Ethanol solvent.

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Identified name** | **Rt\* (min)** | **Peak area (%)** |
| 1 | Tetradecanoic acid, methyl ester | 15.12 | 2.16 |
| 2 | Pentadecanoic acid, methyl ester | 16.43 | 0.60 |
| 3 | Hexadecanoic acid, methyl ester | 17.68 | 24.22 |
| 4 | Hexadecanoic acid, 15-methyl-, methyl ester | 18.90 | 0.68 |
| 5 | Olealdehyde, dimethyl acetal | 20.16 | 0.68 |
| 6 | 17-Octadecenoic acid, methyl ester | 20.39 | 46.32 |
| 7 | 9,12-Octadecadienoic acid (Z,Z)-,methyl ester | 20.59 | 0.52 |
| 8 | 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- | 20.95 | 2.22 |
| 9 | 9-Octadecenoic acid (z)-, ethyl ester | 21.09 | 0.65 |
| 10 | Methyl 10-trans,12-cis-octadecadienoate | 21.90 | 0.79 |
| 11 | Methyl 18-methylnonadecanoate | 22.60 | 0.79 |
| 12 | Aspidospermidine, 1,2-didehydro-, (5à,12á,19à)- | 24.64 | 11.39 |
| 13 | 2-Cyclopenten-1-one,2,5,5-trimethyl | 26.35 | 0.61 |
| 14 | Squalene | 26.54 | 1.47 |
| 15 | Benz[c]acridine, 8,9,10,11-tetrahydro-7-methyl- | 27.01 | 0.40 |
| 16 | Quebrachamine | 27.1 | 1.69 |
| 17 | Akuammilan-17-oic acid, methylester | 27.42 | 0.74 |
| 18 | Aspidospermidine-3-carboxylic acid, methyl ester, (2á,3á,5à,12á,19à)- | 27.57 | 0.68 |
| 19 | Akuammilan-17-oic acid, methylester | 28.44 | 3.44 |

Table 3. Phytochemical compounds of *Rhazya* *stricta* extracted by Methanol solvent.

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Identified name** | **Rt\* (min)** | **Peak area (%)** |
| 1 | Cyclohex-1-enyl-dimethyl- amine  | 7.76 | 1.54 |
| 2 | Pyridine, 3-ethyl  | 8.92 | 5.63 |
| 3 | Octadecanoic acid, 9,10-epoxy-, isopropyl ester | 28.25 | 0.39 |
| 4 | 2,5-Cyclohexadiene-1,4-Dione, 2-(methoxymethyl)-3,5 Dimethyl- | 28.48 | 0.99 |
| 5 | 1,3,4,5-Terahydroxy-cyclohexanecarboxylic acid  | 33.22 | 1.03 |
| 6 | Mome Inositol | 36.50 | 5.26 |
| 7 | Halofantrine | 42.93 | 0.51 |
| 8 | Aspidospermidine, 1,2-didehydro-,(5à,12á,19à) | 45.79 | 28.37 |
| 9 | 3-Acetyl-9-Phenyl-1-Aza-7 Oxabicyclo[4.3.0] non-2-ene | 47.52 | 0.82 |
| 10 | 2-Ethyl-3-[2'-3"-Ethylpiperiduethyl]Indole  | 48.80 | 1.41 |
| 11 | 2,1-Benzisoxazole, 6-(1,1-dimethylethyl)-5-methoxy-4-nitro | 50.26 | 0.28 |
| 12 | 3-cyano-5,5-dimethyltetrafura N-2-one | 50.41 | 3.47 |
| 13 | Aspidospermidine-3-carboxylic acid, 2,3-didehydro-, methylester, (5à,12á,19à)- | 51.18 | 0.56 |
| 14 | Vallesamidine | 51.32 | 0.29 |
| 15 | Eburnamenine | 51.77 | 1.02 |
| 16 | Benz [C]acridine,8,9,10,11-tetrahydro-7-methyl- | 51.87 | 1.44 |
| 17 | Quebrachamine | 52.14 | 11.96 |
| 18 | Clindamycin | 52.39 | 4.43 |
| 19 | 1,3,9,10-tetramethoxypyrrolo[3,2,1-de] phenanthridin-7-one | 53.25 | 0.49 |
| 20 | 2-ethyl-3-[2'-3"-ethyl piperidu ethyl] indole | 53.64 | 1.70 |
| 21 | Aspidospermidine-3-carboxylic acid,methyl ester, (2á,3à,5à,12á,19à)- | 53.81 | 14.27 |
| 22 | 4H-1,3-benzodioxino[4,5-AB]quinolizin-2-one,dodecahydro-12B-methyl- | 54.76 | 5.04 |
| 23 | Akuammilan-17-oic acid, methyl ester | 55.79 | 1.72 |
| 24 |  Vitamin E | 58.71 | 0.32 |
| 25 | 2-Cyclohexyl-4-hydroxymethyl-1-oxa-2-az a-spiro[5.5]undecane-3 carbonitrile, TMS derivative | 62.36 | 1.75 |

Table 4. Phytochemical compounds of *Rhazya* *stricta* extracted by Diethyl ether solvent.

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Identified name** | **Rt\* (min)** | **Peak area (%)** |
| 1 | Propane, 2-methoxy-2-methyl- | 2.56 | 2.18 |
| 2 | 15-methyltricyclo [6.5.2(13,14). 0 (7,15)]pentadeca-1,3,5,7,9,1 1,13-heptene | 13.11 | 0.43 |
| 3 | Tetradecanal | 13.77 | 2.11 |
| 4 | 3,7,11,15-Tetramethyl-2-hexadecen-1-ol | 15.54 | 0.95 |
| 5 | aR-Turmerone | 16.23 | 0.58 |
| 6 | Hexadecanal | 16.45 | 2.62 |
| 7 | Hexadecanoic acid, methyl ester | 17.69 | 1.37 |
| 8 | 9-Octadecenal, (Z)- | 19.01 | 1.20 |
| 9 | Phytol | 20.12 | 1.85 |
| 10 | 17-Octadecenoic acid, methyl ester | 20.35 | 2.07 |
| 11 | Phthalic acid, butyl hex-3-yl ester | 20.75 | 1.21 |
| 12 | Aspidospermidine, 1,2-didehydro-,(5à,12á,19à)- | 24.63 | 26.76 |
| 13 | Phthalic acid, di(2-propylpentyl) ester | 25.61 | 9.19 |
| 14 | Aspidospermidine | 26.35 | 0.92 |
| 15 | Squalene | 26.55 | 22.49 |
| 16 | 1H-Indolo [3,2,1-de] pyrido [3,2,1-ij] [1,5] naphthyridine,13a-ethyl-2,3,5,6,13a,13b-hexahydro- | 27.01 | 0.88 |
| 17 | Quebrachamine | 27.10 | 5.49 |
| 18 | Dotriacontane | 27.30 | 1.91 |
| 19 | Akuammilan-17-oic acid, methyl ester | 27.42 | 0.65 |
| 20 | Aspidospermidine-3-Carboxylic Acid, 2,3-didehydro-, methyl ester, (5à,12á,19à)- | 27.58 | 2.15 |
| 21 |  Isochiapin B | 27.88 | 0.49 |
| 22 | Yohimban-17-one | 28.97 | 0.77 |
| 23 | Vitamin E | 29.52 | 2.16 |
| 24 | Hexaphenylcyclotrisiloxane | 31.68 | 0.57 |

Table 5. Phytochemical compounds of *Rhazya* *stricta* extracted by Chloroform-Methanol solvent.

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Identified name** | **Rt\* (min)** | **Peak area (%)** |
| 1 | Decanoic acid, methyl ester | 9.29 | 0.71 |
| 2 | Dodecanoic acid, methyl ester | 12.33 | 1.46 |
| 3 | Methyl tetradecanoate | 15.15 | 6.03 |
| 4 | Pentadecanoic acid, methyl ester | 15.85 | 0.26 |
| 5 | Tetradecanoic acid, 12-methyl-, methyl ester | 16.01 | 0.23 |
| 6 | Hexadecanoic acid, methyl ester | 17.12 | 0.23 |
| 7 | Methyl trans-4-(2-nonylcyclopentyl)butanoate | 20.12 | 0.32 |
| 8 | Methyl stearate | 20.54 | 47.55 |
| 9 | 9,12,15-Octadecatrienoic acid,methyl ester, (Z,Z,Z)- | 20.99 | 0.39 |
| 10 | Oxiraneoctanoic acid,3-octyl-, methyl ester,trans- | 21.57 | 0.30 |
| 11 | 9,12-Octadecadienoic acid (Z,Z)-,methyl ester | 21.91 | 0.56 |
| 12 | Eicosanoic acid, methyl ester | 22.61 | 0.76 |
| 13 | Aspidospermidine, 1,2-didehydro-, (5à,12á,19à)- | 24.64 | 1.53 |
| 14 | Tetracosanoic acid, methyl ester | 25.76 | 0.26 |
| 15 | cyclohexanone,2,2-dimethyl- | 27.57 | 0.27 |
| 16 | Akuammilan-17-oic acid, methyl ester | 28.44 | 0.66 |

Table 6. Phytochemical compounds of *Rhazya* *stricta* extracted by Ethyl Acetate solvent

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Identified name** | **Rt\* (min)** | **Peak area (%)** |
| 1 | 5-(4-morpholinyl)-2-nitro-Benzenamine | 4.60 | 0.13 |
| 2 | 12,15-Octadecadiynoic acid, methyl ester | 5.57 | 0.12 |
| 3 | Benzene, (2-decyldodecyl)- | 5.65 | 0.10 |
| 4 | Ethyl iso-allocholate | 6.73 | 0.25 |
| 5 | Tetradecane | 8.18 | 0.99 |
| 6 | Pyridine, 3-ethyl- | 9.03 | 4.01 |
| 7 | 4-Benzyloxy-6-hydroxymethyl-tetrahydropyran-2,3,5-triol | 10.34 | 0.16 |
| 8 | 12,15-Octadecadiynoic acid, methyl ester | 12.66 | 0.16 |
| 9 | 9-Octadecynoic acid, methyl ester | 14.14 | 0.11 |
| 10 | Z,Z,Z-4,6,9-Nonadecatriene | 15.52 | 0.18 |
| 11 | 3-(5-Benzyloxy-3-methylpent-3-enyl)-2,2-dimethyloxirane | 16.97 | 0.12 |
| 12 | 11,13-Dihydroxy-tetradec-5-ynoic acid, methyl ester | 17.43 | 0.22 |
| 13 | 7-Methyl-Z-tetradecen-1-ol acetate | 19.11 | 0.19 |
| 14 | 1,3,5-triazine-2,4-diamine, 6-chloro-N-ethyl- | 20.87 | 0.11 |
| 15 | 12-Methyl-E,E-2,13-octadecadien-1-ol | 21.01 | 0.12 |
| 16 | 2,5-Octadecadiynoic acid, methyl ester | 21.18 | 0.08 |
| 17 | 1-Heptatriacotanol | 21.77 | 0.15 |
| 18 | 5,8,11,14-Eicosatetraenoic acid, methyl ester, (all-Z)- | 22.52 | 0.09 |
| 19 | Pregn-5-ene-3,11-dione,17,20:20,21-bis[methylenebis(oxy)]-, cyclic 3-(1,2-ethanediyl acetal) | 24.03 | 0.20 |
| 20 | 1-Oxaspiro[2.5]octane,5,5-dimethyl-4-(3-methyl-1,3-butadienyl)- | 24.83 | 0.13 |
| 21 | 10-Heptadecen-8-ynoic acid, methyl ester, (E)- | 25.66 | 0.18 |
| 22 | Cholestan-3-ol, 2-methylene-, (3á,5à)- | 26.06 | 0.48 |
| 23 | Lactaropallidin | 26.50 | 0.12 |
| 24 | Cyclopentanol, 3,3,4-trimethyl-4-p-tolyl-, (R,R)-(+)- | 27.10 | 0.09 |
| 25 | 2(4H)-Benzofuranone,5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, (R)- | 27.36 | 1.88 |
| 26 | Neophytadiene | 28.25 | 1.55 |
| 27 | 1-Heptatriacotanol | 29.15 | 0.35 |
| 28 | aR-Turmerone | 29.39 | 2.10 |
| 29 | Curlone | 29.83 | 0.48 |
| 30 | 2-Pentadecanone, 6,10,14-trimethyl- | 30.14 | 1.79 |
| 31 | Isochiapin B | 34.27 | 0.27 |
| 32 | Oleic Acid | 39.22 | 2.16 |
| 33 | Cyclopropanebutanoic acid,2-[[2-[[2-[(2 pentylcyclopropyl)methyl]cyclopropyl]methyl]cyclopropyl] methyl]-, methyl ester | 41.71 | 0.30 |
| 34 | Aspidospermidine, 1,2-didehydro-, (5à,12á,19à)- | 45.75 | 6.05 |
| 35 | Tetraneurin - A – diol | 46.21 | 0.23 |
| 36 | Ethyl iso-allocholate | 50.24 | 0.19 |
| 37 | Linoleic acid ethyl ester | 50.50 | 0.22 |
| 38 | Quebrachamine | 52.12 | 0.94 |
| 39 | N-Ethyl-desoxy-veratramine | 53.78 | 3.11 |
| 40 | Aspidofractinine, 3-oxo- | 54.74 | 2.04 |
| 41 | Stigmast-5-en-3-ol, (3á,24S)- | 56.11 | 0.42 |
| 42 | Trilinolein | 57.88 | 0.43 |
| 43 | Vitamin E | 58.69 | 1.94 |

Table 7. Comparison of phytochemical compounds of *Rhazya* *stricta* extracted by various solvent.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Ethyl acetate extract | Chloroform-Methanol | Diethyl ether | Methanol | Ethanol | Bioactive compounds  | Kind |
| Area(%) | R T | Area(%) | R T | Area(%) | R T | Area(%) | R T | Area(%) | R T |
|  |  |  |  |  |  | 1.54 | 7.76 |  |  | Cyclohex-1-enyl-dimethyl-amine |  |
|  |  |  |  |  |  | 5.63 | 8.92 |  |  | Pyridine, 3-ethyl- |  |
|  |  |  |  |  |  | 0.29 | 51.32 |  |  | Vallesamidine |  |
|  |  |  |  | 0.92 | 27.1 | 11.96 | 52.14 |  |  | Quebrachamine |  |
|  |  |  |  |  |  | 4.43 | 52.39 |  |  | Clindamycin |  |
|  |  | 1.53 | 24.6 | 26.76 | 24.6 |  |  |  |  | Aspidospermidine, 1,2-didehydro-,(5à,12á,19à)- | Alkaloids |
|  |  |  |  | 0.92 | 26.3 |  |  |  |  | Aspidospermidine |  |
|  |  |  |  | 0.49 | 27.8 |  |  |  |  | Isochiapin B |  |
|  |  |  |  | 0.77 | 28.9 |  |  |  |  | Yohimban-17-one |  |
|  |  | 0.66 | 28.4 |  |  |  |  |  |  | Akuammilan-17-oic acid, methyl ester |  |
|  |  | 0.26 | 25.7 |  |  |  |  | 2.16 | 15.12 | Tetradecanoic acid, methyl ester |  |
|  |  |  |  |  |  |  |  | 0.60 | 16.43 | Pentadecanoic acid, methyl ester |  |
|  |  | 0.23 | 17.1 | 1.37 | 17.6 |  |  | 24.22 | 17.68 | Hexadecanoic acid, methyl ester |  |
|  |  |  |  | 2.07 | 20.35 |  |  | 46.32 | 20. 39 | 17-Octadecenoic acid, methyl ester |  |
| 0.12 | 5.57 | 0.56 | 21.91 |  |  |  |  | 0.52 | 20. 59 | 9,12-Octadecadienoic acid (Z,Z)-, methyl ester | Fatty acid |
|  |  | 0.39 | 20.99 |  |  |  |  | 2.2 | 20.95 | 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- |  |
|  |  |  |  |  |  | 1.72 | 55.79 |  |  | Akuammilan-17-oic acid, methyl ester |  |
|  |  |  |  | 1.85 | 20.1 |  |  |  |  | Phytol | Triterpene |
|  |  |  |  | 22.49 | 26.5 |  |  |  |  | Squalene |  |
|  |  |  |  | 1.21 | 20.7 |  |  |  |  | Phthalic acid, butyl hex-3-yl ester | Antimicrobial |
|  |  |  |  | 9.19 | 25.6 |  |  |  |  | Phthalic acid, di(2-propylpentyl) ester |  |
| 1.94 | 58.6 |  |  | 2.16 | 29.5 |  |  |  |  | Vitamin E | Vitamin E |
| 0.42 | 56.1 |  |  |  |  |  |  |  |  | Stigmast-5-en-3-ol, (3á,24S)- | Steroid |
| 0.48 | 26.1 |  |  |  |  |  |  |  |  | Cholestan-3-ol, 2-methylene-, (3á,5à)- |
| 0.25 | 6.73 |  |  |  |  |  |  |  |  | Ethyl iso-allocholate |
|  |  |  |  |  |  | 4.43 | 52.39 |  |  | Clindamycin | Antibiotic |

|  |  |
| --- | --- |
|  |  |
|  |  |