DIVERSE RESOURCING OF *NERIUM INDICUM* LEAVES FOR BIO-UTILIZATION

by

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Original scientific paper
https://doi.org/10.2298/TSCI190603052M

Nerium indicum is an ornamental plant that is widely distributed in tropical and subtropical regions wordwide. It has toxic and medicinal properties which is closely related to the bioactive ingredients contained in Nerium indicum. In our research, the leaves of Nerium indicum was used as raw materials to study the chemical constituents and their effects. The chemical constituents of the leaves were analyzed by FT-IR and GC-MS with alcohol, benzene and acetone as organic solvents. A total of 73 compounds were obtained by acetone organic solvent, 25 compounds were extracted from benzene and 146 compounds were obtained from alcohol. Rich bioactive and bioenergy components were found in all three kinds of extract, suggesting that Nerium indicum leaves are of great significance for the diverse resourcing of bio-utilization including biomedicine, bioenergy, aroma, food additives .

Key words: *Nerium indicum, chemical compositions, bioenergy*

Instructions

Nerium indicum is widely planted in China, particularly in the southern provinces. Due to its ornamental, they are mainly planted in parks, beauty spot, roads, rivers and lakes. As we know, the leaves, bark, roots, flowers and seeds of *Nerium indicum* contain a variety of carbohydrates, which are very toxic which can kill humans and animals by feeding [1]. At the same time, the whole body of *Nerium indicum* is a treasure and it has abundant biomass resource. The leaves is helpful in reinforcing diuresis and expectorate, and it can also kill insects. The root and the skin can be used to make cardiotonic. Moreover, the body of *Nerium indicum* have function of dispersing blood stasis, relieving pain and detoxification. It also can be used against asthma, sheep madness, heart failure, alopecia areata and so on [2].

The toxicity and efficacy of *Nerium indicum* leaves are closely related to the chemical constituents contained in it. In order to study the chemical constituents of *Nerium indicum* leaves, the extracts with alcohol, benzene and acetone as organic solvents were analyzed by FT-IR and GC-MS respectively [3].

Materials and methods

Experimental materials

As you can see from the fig. 1, the leaves of *Nerium indicum* were collected in Henan Agricultural University. The samples were cleaned, and placed at room temperature (25 °C) to

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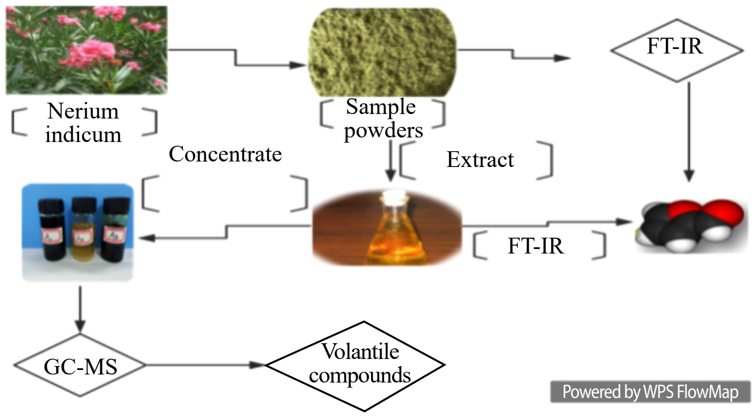

Figure 1. The composition analysis route of the leaves of the *Nerium indicum*

dry naturally, then put them in a grinder (FW100) [3]. The 200 mesh powder was screened, and then dried and preserved. Each sample was extracted with ethanol, alcohol and acetone.

Methods

Extraction by three solvents

The *Nerium indicum leaves* are extracted with ethanol, benzene and acetone, each group of samples was 15 g and organic solvent was 300 mL. The sample was soaked at room temperature for 8 hours, distilled with ethanol, benzene and acetone at 80 °C, 80 °C and 56 °C for 4 hours, then filtered with qualitative filter paper under vacuum, then evaporated at about 50 ℃ and concentrated to about 20 mL, filtered again with needle tube and filter head, sealed into injection bottle, extracted residue and dried,vand all extracts were stored at 4 °C [4, 5].

The FT-IR analysis

The environmental conditions such as power supply, temperature and humidity should be checked before starting. When the voltage is stable, the room temperature is 25 °C and the humidity is less than 60%. After 200-mesh screening and extraction of the sample [6], 200 mg of potassium bromide was added to agate mortar with smooth surface, and solid samples having 0.5-2 mg was mixed with potassium bromide in mortar completely and quickly, and then put into a tablet press for tableting. As a liquid solution, the extract is placed directly in a tablet press for tableting. The pressed samples were tested by Fourier transform infrared spectroscopy at wave lenght from 4000 to 400 cm^{-1} [5-7].

The GC-MS analysis

The GC-MS analysis conditions. Chromatographic conditions: The inlet temperature is 250 °C, the Injection volume is 1 μL, Heating process: The initial temperature is at 50 ℃ without reservation, and then 5 °C/min heat up to 130 °C without reservation, next 3 °C/min to 300 °C with 5 min. The chromatographic column is quartz capillary column (30 mm × 0.25 mm × × 0.25 μm). Mass spectrometry condition: The interface temperature is 250 °C, column flow 1.0 mL/min, split ratio 20:1, the carrier gas was higher helium. Mass spectrometry is carried out under the following conditions: the ionization mode was EI. The electron energy was 70 eV, the ion source temperature was 230 °C [5, 8], the quadrupole temperature is 15 °C, the scanning starting point is 30-600 °C, qualitative search on 7n.1 standard spectrum and computer [5, 9].

Results and analysis

Chemical group change regulation during extractions

According to the infrared functional group comparison table: on the left side of fig. 2, you can see that there are five obvious peaks. The strongest peak appears at 3410 cm^{-1}, which is caused by the expansion vibration of the intermolecular hydrogen bond O-H, which indicates the existence of alcohols and phenols. There is peak overlap at 2910 cm^{-1}, which is due to the extensive absorption of O-H, which indicates the existence of carboxylic acid. The weak peak appeared at 2848 cm^{-1}, which was caused by the expansion of C-H, which confirmed the existence of aldehyde and ketone, and also appeared at 1750 cm^{-1}, which may be caused by the tensile vibration of C=O. There are also peaks at 1630 cm^{-1}, usually due to NH deformation and vibration, indicating the presence of amine compounds [10, 11]. The peak at 1050 cm^{-1} is caused by the telescopic vibration of C-O, which represents the existence of esters. The presence of a peak at 590 cm^{-1} can be inferred to the presence of a halogen.

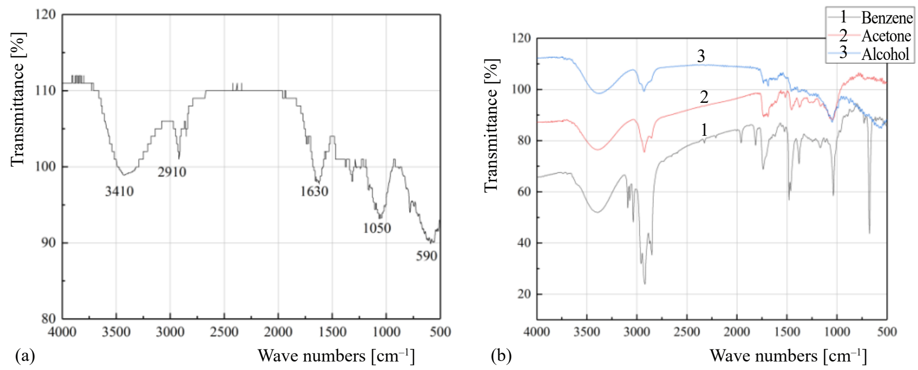

Figure 2. (a) The FT-IR spectra of the original powder, (b) The FT-IR spectra of extracts benzene, acetone, and alcohol

As you can see from the right side of fig. 2. First of all, at 3410 cm^{-1}, the absorption peak intensity of the three kinds of extract is similar, which means that the content of alcohol in the three kinds of extract is relatively similar. Secondly, the absorption peak intensity of benzene extract at 1630 cm^{-1} was higher than that of ethanol extract and acetone extract, which indicated that the content of amine in benzene extract was higher than that in ethanol extract and acetone extract. Thirdly, the absorption intensity of ethanol extract at 2910 was higher than that of the other two, which indicated that the content of carboxylic acid in ethanol extract was higher than that of the other two.

The infrared spectra of the original solid samples are similar to those of the original liquid, in which the trend of benzene extract is relatively large, the general trend of ethanol extract and acetone extracts is mild, and the trend of alcohol is the best. Ethanol is thus the best solvent to extract compounds from the leaves of the *Nerium indicum*. The right side of fig. 2, The general trend of infrared spectra drawn from the three kinds of oleander leaves extract is the same, among which the ethanol extract and the acetone extract are similar, which indicates that the acetone extract and the ethanol extract contain the same compounds.

Temperament analysis

Twenty-five compounds were detected from 37 peaks of benzene extract from oleander peach leaves, fig. 3. The bioenergy has been found in benzene extract of *Nerium indicum* leaves: o-Xylene can be used as aviation gasoline additive [12], 2-Ethylhexanol can be used as fuel additive for diesel engine, and triethylamine can be directly used as high energy fuel [13, 14].

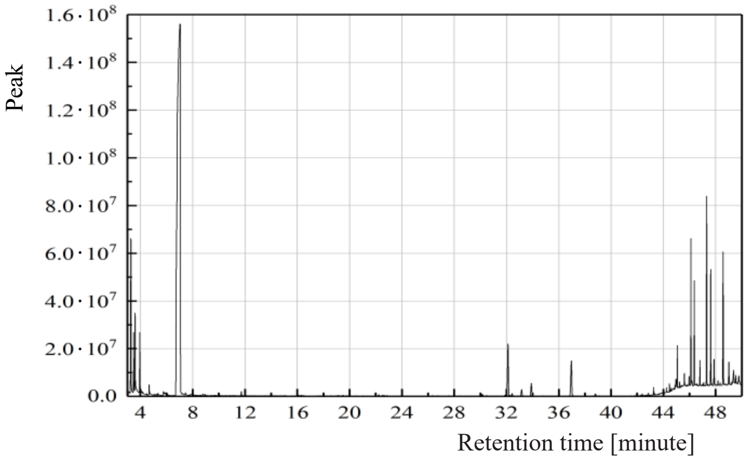

Figure 3. Ion chromatogram of the benzene extract from *Nerium indicum* leaves GC-MS/MS

Many chemical raw materials were also detected in the benzene extract of *Nerium indicum* leaves. Diacetone alcohol can be used as high boiling point solvent, paint diluent, wood colorant, rust remover and dye. Dibutyl phthalate is mainly used as plasticizer for nitrification fiber, acetate fiber, polyvinyl chloride and so on. The P-xylene is an important organic chemical raw material [15, 16].

Many biomedicines were also detected in the benzene extract of *Nerium indicum* leaves. Trans-squalene is a nutritional drug, internal administration can treat anemia, diabetes, liver cirrhosis, cancer, *etc.*, external application can treat tonsillitis, rheumatism, neuropain and so on. Squalene can be used as a nutritional supplement to improve liver function and tissue activity [17-19]. Uvaol has a certain bacteriostatic effect, and now a western medicine composition for the treatment of tonsillitis has been developed.

A small amount of cosmetics, food additives and spices were also detected in the benzene extract of *Nerium indicum* leaves: DL-α-Tocopherol has anti-aging and antioxidant effects, so it can be used as raw materials for cosmetics [20]. The 1-dodecanol and 1-undecanol are allowed edible spices, mainly used to prepare lemons, oranges, coconut and pineapple. They can also be used in evening jade, clover, boll orchid, violets, acacia, shy flowers, roses, narcissus, wood fragrance and other fantasy flavors.

The 73 compounds were detected from 88 peaks of acetone extract from *Nerium indicum* leaves, fig. 4. O-xylene and p-xylene can also be detected in acetone extract of oleander leaves, which can be used as bioenergy.

Many chemical raw materials were also detected in acetone extract of *Nerium indicum* leaves. Phenol is an important organic synthetic raw material, which can be used to make phenol-formaldehyde resin, phenolphthalin, picric acid, salicylic acid, NA and other chemicals.

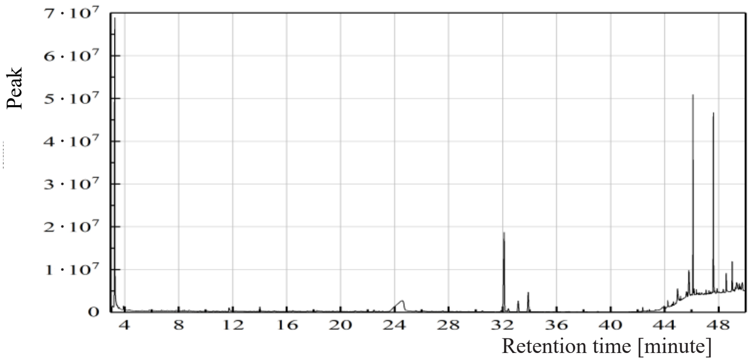

Figure 4. Ion chromatogram of acetone the extract from *Nerium indicum* *l*eaves GC-MS/MS

The most important use of styrene is as a monomer of synthetic rubber and plastics, which can be used to produce SBR, polystyrene, foam polystyrene, and a small amount of styrene is also used as intermediates such as spices [21]. The M-Xylene is used in the production of isophthalic acid, m-methylbenzoic acid, isophthalic nitrile, *etc*. It can also be used as medicine, dye, perfume, and color film [22].

Many biomedicines were also detected in the benzene extract of *Nerium indicum* leaves.for example, the Wintergreen Oil has anti-inflammatory and analgesic effect, and is widely used in the joint muscle pain-relieving paste, the tincture and the oil agent. Phyltol is a basic raw material for producing vitamin K_1 and vitamin E. Betulin, as a biological agent, has a great potential as a biological agent in the treatment of HIV and cancer, and is effective by interfering with the post-phase of the viral life cycle, it is also an effective anti-tumor drug, which can lead to the initiation of self-destruction of some types of tumor cells, and can slow down several types of tumor cell growth. Lupol has anti-tumor, anti-inflammatory and antioxidant effects.

A small amount of cosmetics were also detected in acetone extract from *Nerium indicum* leaves, undecanal and dodecyl aldehyde have the aroma of rose, flower, fruit and sweet orange in the diluted state. It can be used for flower flavor extraction and adjustment, which can be used as raw material composition of cosmetics. Undecan-4-olide has a pleasant, iris-like sweet-fat fragrance, and can be used as a soap and a daily cosmetic.

A plurality of food additives and a perfume are also detected in the extract of the *Nerium indicum* leaves acetone. Styrene, (-)-myrtenol, hydroxycitronellol, Phenethyl alcohol were also detected in acetone extract of oleander leaves as food spices specified by GB2760-96, and all of them had special flavor, so they could be used as flavors.

The 146 compounds were detected from 169 peaks of ethanol extract from *Nerium indicum* leaves, fig. 5. A small amount of bioenergy and cosmetic is found in the ethanol extract of the leaf of the *Nerium indicum*. For example, vanillin has pod orchid bean aroma and strong milk fragrance, which is widely used in a variety of flavoring substances that need to increase milk flavor, such as toothpaste, soap, perfume cosmetics, daily cosmetics and so on, which play the role of incense and odour.

A large number of chemical raw materials have been found in the ethanol extract of *Nerium indicum* leaves. Benzyl alcohol is used as preservative for ointment, fiber, nylon silk and plastic film desiccant, polyvinyl chloride stabilizer, photographic developer, cellulose

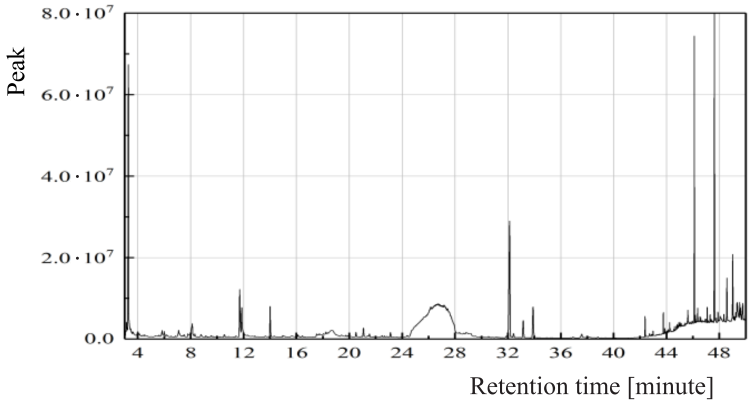

Figure 5. Ion chromatogram of alcohol the extract from *Nerium indicum* leaves GC-MS/MS

acetate, ink, coating, paint [23]. Oleic acid is an organic chemical raw material used to make soap, lubricant, flotation agent, ointment and oleate, and also is a good solvent for fatty acids and oil-soluble substances [24]. 2, 2 -Methylen Ebis (6-tert-butyl-4-methylphenol) is a kind of high efficiency and non-polluting antioxidant, which is widely used in synthetic resin, synthetic rubber and natural rubber. it has the characteristics of excellent anti-thermal oxygen aging, non-discoloration of products and so on.

Many biomedicines have also been detected in the ethanol extract of *Nerium indicum* leaves, such as EPA can reduce blood lipid, blood pressure and blood glucose, prevent and improve arteriosclerosis and prevent hypertension. The 4, 5 (Dimethyl1), 3 (dioxol) 2 × one can be used as the intermediate of olmesartan, an antihypertensive drug. Beta-Sitosterol has the effects of reducing cholesterol, relieving cough, expectorant, inhibiting tumor and repairing tissue. It can be used in type II hyperlipidemia, atherosclerosis and chronic tracheitis, early cervical cancer and skin ulcers. Stigmasterol is mainly used as raw material for the synthesis of steroid hormones, and can also be used as raw material for the production of vitamin D_3.

Many food additives and spices were also detected in the ethanol extract of oleander leaves. For example: Palmitic acid ethyl ester is an edible flavor allowed by GB2760-96. It can be used to prepare cream, beef fat, milk, pork and fish, and flavor for spices. Palmitic acid has special aroma and taste. According to GB2760-89 regulations of our country, it can be used to prepare all kinds of edible spices, as well as raw materials for defoamer and other food additives.

The bioenergy and bioactive substances in the leaf extract of different Nerium indicum leaves

It can be seen from fig. 6, that there are a large number of chemical raw materials and biomedicine in the three different extracts, all of which reach more than 40%, among which the benzene extract is the highest in the chemical raw material, indicating that the benzene extract is the most important raw material in the chemical industry. For bioenergy, the content of the three is not high, among which the bioenergy in benzene extract is relatively high. For cosmetics, acetone extract and benzene extract have the same content, but all of them are lower than ethanol extract. In biomedicine, food additives and aroma components, acetone extract was lower than the other two, but ethanol extract and acetone extract had no significant difference.

The functional groups of three kinds of Nerium indicum leaves extract

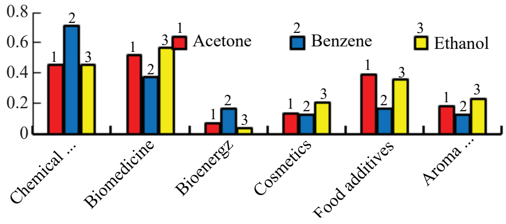

Figure 6. Comparison of non-volatile compounds in three extracts from the leaves of *Nerium indicum* (the same substance can be used for various purposes)

As can be seen from fig. 7, among the three extracts, alcohols are the highest, such as benzyl alcohol, diacetone alcohol, beta-sitosterol, stigmasterol, which are mainly used in chemical raw materials and biomedicine. Alkane is found only in benzene extract. For example, N-heptadecane is a phase change material for gas phase analysis and detection. The N-nonadecane can be used for organic synthesis and chromatographic analysis. Only ethanol extract contains EPA, oleic acid, methyl jasmonate and other carboxylic acid compounds. For example, methyl jasmonate is widely used in artificial jasmine clean oil and jasmine base, but it has not been widely used in fact because of its high price. Aromatic hydrocarbons and olefins contain the least in ethanol. However, ketones, esters and phenols were the highest in ethanol extract. Silicon compounds can be found in acetone extract and benzene extract. Such as dimethyldimethoxysilane as structural control. It can be used instead of hydroxyl silicone oil by improving the machining performance, prolonging the storage time of the mixer. The N-octadecyltrichlorosilane is an intermediate used to produce silicone, which is used to synthesize silicone intermediate and polymer. Mercaptan can be found in acetone extract, such as di-tert-dodecyl disulfide, which can be used in chemical reagents, fine chemicals, pharmaceutical intermediates.

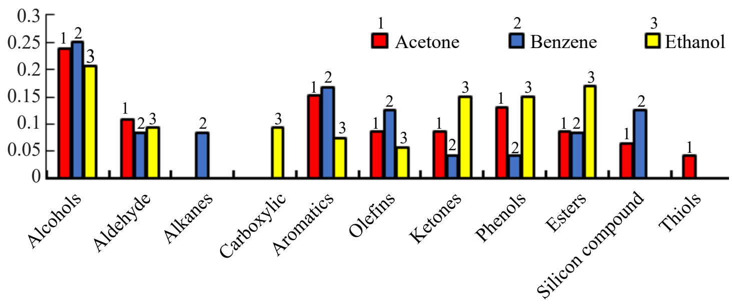

Figure 7. Comparison of functional groups of non-volatile compounds in three kinds of *Nerium indicum* leaves extract

Conclusion and discussion

After extraction with various solvents, the infrared transmittance of the original powder of transparent liquid crystal body showed different degrees of change. The results of infrared spectroscopy show that there are absorption peak between 3500-3000 cm^{-1}, 3000-2750 cm^{-1}, 1750-1500 cm^{-1}, and 1500-1000 cm^{-1}. Compared with the infrared spectra of the three kinds of extract, the general trend of acetone extract is mild, and the overall trend of benzene extract is more tortuous. The trend of alcohol is the best. The results showed that ethanol was the best solvent for extracting compounds from fresh leaves of *Nerium indicum*.

The chemical substances of *Nerium indicum* leaves were analyzed by infrared and gas technology. A total of 49, 25 and 54 compounds were respectively obtained from acetone organic solvent, benzene and alcohol. *Nerium indicum* leaves ethanol extract, benzene extract and acetone extract are rich in bioenergy components, which can be used in chemical and medical raw materials. Leaf ethanol extract, leaf acetone extract and leaf benzene extract have a certain amount of aroma components, which can be used as cosmetics and food additives. It is well known that the *Nerium indicum* has a wide range of toxicity and pharmaceutical value, which is closely related to the bioactive ingredients contained in the leaves. The results are of great significance for the further development of *Nerium indicum* resources.

Acknowledgment

This project was supported by the Project of Henan Provincial Science Research, China (192102110174), and the Talent Project (Dangquan Zhang) of Henan Agriculture University, China.

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Paper submitted: June 3, 2019
Paper revised: August 19, 2019
Paper accepted: September 3, 2019