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Optimization of crude rotenone oil extraction from birbira plant (Milletia ferruginea) seed by Soxhlet and maceration methods

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Abstract

The extraction of active compounds from plant materials is one of the most critical processes in the commercial development of natural products for pharmaceutical, herbicide or pesticide production. The focus of this study was extraction of crude rotenone oil (CRO) from Birbira plant (Milletia ferruginea) seed. The extraction was done using maceration and a Soxhlet extractor using organic solvents, such as ethanol, hexane, and chloroform. For the efficient extraction of crude rotenone oil from Birbira plant seed the effect of parameters, such as extraction method, solvent type, extraction time and size of crushed Birbira seed were investigated. Based on the experimental results obtained Soxhlet extraction method was more efficient (41.6% CRO yield) compared to the maceration extraction method (25.1 % CRO yield). The extraction results for both Soxhlet and maceration methods indicated that chloroform is more efficient compared to ethanol and hexane solvents. On the other hand, the effects of crashed seed size and extraction time on the % CRO yield were also investigated. The results showed that medium size (0.1–0.35 mm) for both Soxhlet and maceration extraction was the optimum size leading to the highest % CRO yield compared to fine and coarse ground seed size. The highest % CRO yield was obtained during 4 to 5 h of Soxhlet extraction and 25 to 30 h of maceration extraction method. Therefore, the Soxhlet extraction method is the fastest and efficient method for the extraction of CRO from plant materials. The characterization of Birbira seed powder and CRO was done with FTIR and Rheology test equipment. The FTIR result revealed that CRO is composed of aliphatic, olefin, polyphenol and alcohol functional groups, in which polyphenol functional groups are the most essential flavonoid components available in crude rotenone oil.

1. Introduction

The majority of people in the world now rely on agricultural products for their livelihood, yet the development and yield of crops are being hindered daily. Farmers frequently anticipate a drastic treatment to make their crops green and healthy in order to boost output when they observe a decline in the yield and production of their crops. Consequently, they begin using chemical pesticides without considering their long-term consequences. Although conventional chemical pesticides have increased food production, they have also had unfavorable effects on humans, the environment, and non-target creatures. The majority of pesticides that are now in use have a propensity to persist in plants for extended periods of time [1]. They also find their way into the food chain through meat and dairy products, where they persist for a considerable amount of time as residue in the ecosystem and soil.

Finding biopesticide made of plant components as an alternative to conventional pesticides is therefore crucial in order to manage pests without compromising agricultural productivity and profitability [2]. Because of the adverse effects of chemical pesticides, eco-friendly management is basically necessary for sustainable crop production. One such eco-friendly method is to use rotenone, which is extracted from plant materials like Birbira plant (Milletia ferruginea) seed, to replace some of the most problematic chemical pesticides currently in use. Rotenone extract, a biopesticide derived from Birbira seeds [3], has the potential to effectively address public apprehension regarding the adverse effects of chemical pesticides on the environment and, eventually, human health due to persistent pesticide contamination of agricultural products.

Birbira plant (Milletia ferruginea) is an indigenous plant species [4] found only in Ethiopia [5] and widely distributed in the country [5]. The active component, rotenone, is known to have pesticidal properties. It has been traditionally used to treat fish poisoning by pulverizing mature pods and seeds and dispersing them evenly throughout the water's surface [5-7]. This dominant compound which is responsible for its poisoning is found in the Birbira seed and stem bark of Milletia ferruginea [6, 7].

Fig. 1. Resu Figure 1: Images of (A) Birbira (Milletia ferruginea) plant, (B) Birbira plant matured seed, and (C) Birbira plant seed.

The most influential parameters for the extraction of active components from plant materials, Birbira plant seed, are type of extraction method employed, solvent type and extraction time. Extraction methods that can be employed to extract rotenone from plant materials include conventional methods such as normal soaking or maceration (stirring soaking) method, Soxhlet method and non-conventional methods such as pressurized liquid extraction method, supercritical fluid extraction method and microwave-assisted extraction method. Bandar et al. [8] concluded that among the conventional extraction methods, Soxhlet extraction method had the highest extraction yield compared to the maceration method. However, compared to other contemporary extraction techniques, maceration has been proposed as a more suitable, affordable, and practical approach for small and medium-sized businesses.

Important characteristics that should be taken into account as one of the factors influencing the extraction yield are the process conditions for each extraction method. Studies on optimization have revealed that the types and strengths of the solvents are the most important parameters in nearly all approaches. Solvent to sample ratio, however, is said to have no discernible impact, indicating that excessive volumes of solvents should be avoided [8]. Temperature, solvents, agitation speed, extraction duration, and other significant parameters may all improve extraction, but if used improperly, they may also degrade the compounds [8]. Thus, when selecting appropriate approaches, it may be prudent to take into account those with the fewest influencing aspects. Consequently, crushed seed size, solvent type, and extraction conditions (time and temperature) should be operated at suitable conditions, which would give the highest rotenone yield. However, the optimal extraction time will depend on the type of solvent used and thus, solvent type to extraction time combination should be optimized.

2. Materials and methods

2.1. Chemicals

The main chemicals (consumables) used in the present research work are cyclohexane (99%), ethanol (99%), and chloroform (99.8%) which were obtained from LOBA Chemie, Laboratory Reagents and Fine Chemicals PVT. LTD.

2.2. Sample preparation

Raw material, ripe Birbira plant seed, was collected from Amhara region (Bahir Dar area) and this raw material was washed and dried before crushing. Then, dried Birbira plant seed was crushed by using grinder and it is separated into fine (0-0.10 mm), medium (0.10-0.35 mm) and coarse (0.35 – 0.50 mm) particle sizes with sieve analysis.

2.3. CRO Extraction by Soxhlet Extractor

The Soxhlet extraction of CRO with different solvents for each crushed Birbira seed powder was performed from 1 to 6 h. Samples of 10 g of crushed Birbira seeds were prepared and put within a thick filter paper thimble that was then inserted into the Soxhlet extractor's primary extraction chamber. The Soxhlet extractor was put onto a flask holding one of the three solvents chosen as a parameter to see the effect of solvents on the extraction. The extraction was done with 100 mL of ethanol, hexane and chloroform solvent and each solvent was heated to reflux. A siphon side arm automatically drained the Soxhlet chamber when it was nearly full, allowing the solvent to return to the distillation flask. This cycle was done repeatedly many times until the desired amount of extract was obtained. The extract from Soxhlet extractor was evaporated in an oven dryer at 65°C to 85°C for 4 to 5 h depending on the type of solvent used and to be removed from the product mixture. The drying process in the oven was repeated more than three times until a constant amount of CRO was obtained after removing the solvent used during extraction.

Fig. 1. General process flow diagram of CRO extraction by Soxhlet extractor.

2.4. CRO Extraction by Maceration

Samples 30 g of crushed Birbira seed were put in a stopped conical flask and macerated for 5 to 30 h at room temperature with periodic stirring using 300 mL of ethanol, hexane, and chloroform solvent. The extract and solvent mixture were then filtered using a muslin cloth and evaporated in an oven dryer at 65 to 85°C based on the boiling points of the solvents used for the maceration extraction. The drying process in the oven was repeated more than three times until a constant amount of CRO was obtained after removing the solvent used during extraction. The crude extract was stored in an ice box with a tightly sealed container, shielded from light, due to its extreme sensitivity to temperature changes. This procedure was repeated by using other Birbira seed powder particle sizes with each solvent to evaluate the best size distribution and solvent for the effective extraction of crude rotenone oil.

2.5. Effect of crushed seed size and extraction time on the extraction yield

The yield of crude rotenone extracted from crushed Birbira seed by Soxhlet apparatus when using different size distributions (coarse, medium and fine size) of crushed seed was calculated as percentage yields of crude oil containing rotenone (g) per 100 g of ground Birbira seed. To determine the percent yield of extracted crude oil, 10 g sample for Soxhlet extraction and 30 g for maceration extraction were used and the difference between the initial mass of the sample and the final mass of the sample obtained after drying was used to calculate the percentage of crude oil extract with the following mathematical expression (Equation (1)).

$$Y(\%) = \frac{m_i - m_f}{m_i} x100 \qquad (1)$$

where, Y (%) is the crude oil yield, m_i is the initial mass of the sample and m_f is the final mass of the sample (raffinate).

3. Results and discussion

3.1. Soxhlet extraction

Effects of extraction time, particle size and type of solvents are shown in Figure 3 (A), (B) and (C) illustrating the effect of particle size and extraction time on the crude rotenone oil yield using ethanol, hexane and chloroform solvents respectively. Kandar [9] indicated that particle size is thought to be an important element in solvent extraction. The crushed seed particle sizes used for this study were 0-0.10 mm, 0.1- 0.35 mm and 0.35 - 0.50 mm.

To observe the effect of time on the crude oil yield 1, 2, 3, 4. 5 and 6 h periods of extraction were performed and the maximum crude oil percentage yield was obtained for the extraction duration of 5 hours. For this optimal time of extraction, oil yield increases when the particle size decreases due to the increase of contact surface area of the particle to the solvent. When the particle size of crushed Birbira seed powder becomes bigger and bigger (> 0.35 mm), the crude rotenone yield decreases as the contact surface area of the particle to solvent decreases. On the other hand, when the Birbira seed powder particles size becomes too fine (< 0.1 mm) the CRO yield decreases as a result of coagulation formation. As shown in Figure 3 (D), the medium particle size (0.1- 0.35 mm) is an optimum crushed seed size giving the highest percentage of crude rotenone oil yield value, which is supported by the research findings of Kandar [9], and the amount of crude oil obtained by using chloroform solvent is higher than that of ethanol and hexane solvents. The result showing the highest extraction yield by using chloroform is also reported by research findings of Sae-Yun et al. [7]

Generally, Soxhlet extraction method is considered more efficient method leading to higher % CRO yield, continuous solvent cycling allows for quicker extractions, fresh solvent minimizes exposure and potential breakdown of compounds (degradation) and the automated process provides more consistent yields between batches. From performed experimental results we can conclude that Soxhlet extraction method is more efficient (higher % CRO yield) compared to maceration extraction methods. This result is strongly supported by the research work of Bandar et al. [8] as they concluded that among the conventional extraction methods, Soxhlet method yielded the highest extraction among the other conventional extraction methods.

3.2. Maceration extraction

The maceration extraction results are presented in Figures 4 (A), (B) and (C) illustrating the effect of particle size and extraction time on the crude rotenone oil yield using ethanol, hexane and chloroform solvents respectively. The crushed seed particle sizes used for this study were 0-0.10 mm, 0.1-0.35 mm and 0.35-0.50 mm. To observe the effect of time on the crude oil yield 5, 10, 15, 20, 25 and 30 h period of extraction was performed and the maximum crude oil percentage yield was obtained for the extraction duration of 25 h. For this optimal time of extraction, oil yield increases when the particle size decreases due to the increase of contact surface area of the particle to the solvent.

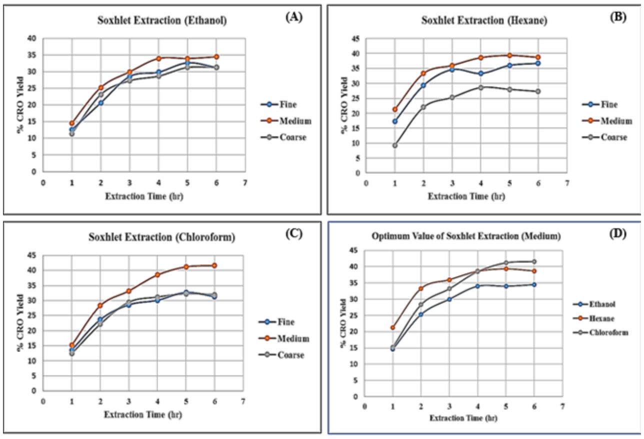

Fig. 3. Effect of particle size and extraction time on percentage of crude rotenone oil yield by Soxhlet extraction method using different solvents (A) ethanol, (B) hexane, (C) chloroform and (D) Soxhlet extraction optimum value.

As shown in the Figure 4 (D), the medium particle size (0.1 – 0.35 mm) is an optimum crushed seed size resulting in the highest % CRO yield value of maceration extraction and its value obtained by using chloroform solvent is higher than that of ethanol and hexane solvents. It is observed that, maceration extraction of CRO from fine seed powder resulted in the higher yield at the beginning of extraction compared to coarse and medium size, but the CRO yield from medium size dominates after 20 h of extraction. This is due to the large surface contact area to volume ratio of fine particles as reported by (Kandar, 2021) leading to higher yield initially and after 20 h it becomes lower and lower because of coagulation of particles and difficulty of separation of CRO by filtration from the mixture.

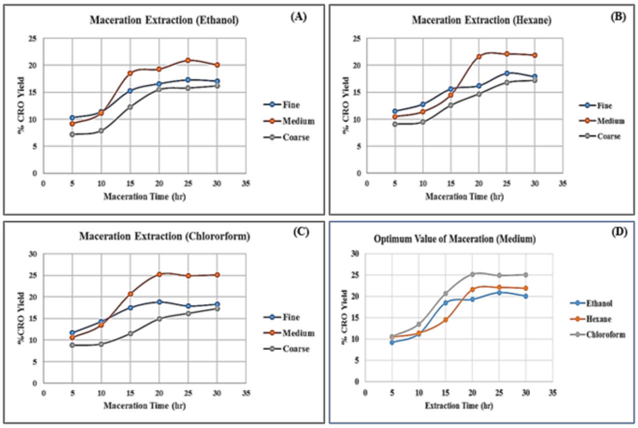

Fig. 1. Effect of particle size and extraction time on percentage of crude

rotenone oil yield by maceration extraction method using different solvents (A) ethanol, (B) hexane, (C) chloroform and (D) maceration extraction optimum value.

3.3. FTIR analysis

FTIR analysis spectra results of CRO extract from medium size Birbira seed powder are illustrated in Fig. 5, showing different functional groups, which are essential components of CRO. The components of CRO functional groups observed from the FTIR analysis spectrum at different wavelengths include; aliphatic ester groups (Propyl acetate and Butyl acetate), aliphatic functional groups, olefin functional groups (1-hexene, trans-2-hexene and cis-2-octene), and alcohol and phenol functional groups [10, 11].. The availability of phenolic components in plant materials as pesticidal compounds was reported in research findings reported by Jose et.al. [12].

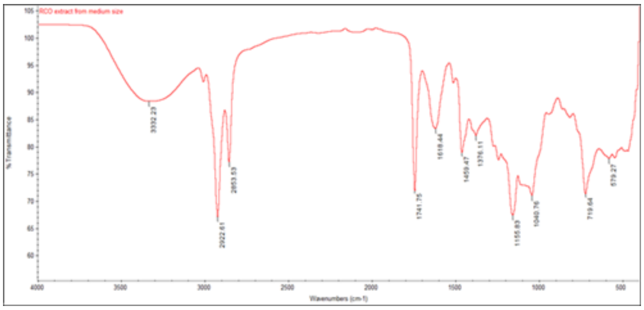

Fig. 5. FTIR analysis results of crude rotenone oil extracted from medium particle size of Birbira seed powder by Soxhlet extractor with chloroform solvent.

From general interpretation of the FTIR spectrum the following functional groups in a molecule were identified in the 4000-400 cm^{-1} wave number region. The large peak at 3332 cm^{-1} is due to O-H stretching, from alcohols or carboxylic acids. The peaks around 2922 and 2853 cm^{-1} are due to C-H stretching, which is found in most organic molecules [13-16].

The peak at 1742 cm^{-1} is indicative of C=O stretching of a ketone or aldehyde. The peak at 1618 cm^{-1} could be associated with the C=C stretching of an alkene or aromatic ring. Ami et al. [14], Kamble and Gaikwad [17], and Mekonnen [18] indicated that there is an asymmetric stretching vibration of CH_3 at 2975-2950 cm^{-1} and an absorption of CH_2 at roughly 2930 cm^{-1}. At 2885-2865 cm^{-1}, symmetric CH_3 vibration takes place, and at roughly 2870-2840 cm^{-1}, CH_2 absorption takes place. The vibration of asymmetric deformation of CH_3 occurs at 1470-1440 cm^{-1}. At 1490-1440 cm^{-1}, this band overlaps with the CH_2 scissor vibration. At 1390-1370 cm^{-1}, the symmetric CH_3 vibration takes place. One can determine the proportions of the asymmetric CH_3 and CH_2 scissor bands in the molecule by comparing their respective intensities [19].

The symmetric CH_3 vibration, which happens around 1390-1370 cm^{-1}, splits into two bands when there are several methyl groups on a single carbon [20, 21]. Three methyl groups on one carbon (t-butyl) result in the appearance of two bands: one near 1365 cm^{-1} and the other, weaker, near 1390 cm^{-1}. When two methyl groups are present on an isopropyl single carbon, bands with almost similar intensities appear at 1390 and 1365 cm^{-1}. Bands around 1255 and 1210 cm^{-1} indicate the presence of the t-butyl group, whereas bands around 1170 and 1145 cm^{-1} indicate the presence of the isopropyl group. In the case of four or more consecutive CH_2, a rocking absorption with a center of 720 cm^{-1} is observed. When the number of neighbouring methylene groups approaches ten, this absorption divides into two bands [21-24].

Alkenes, another name for olefin functional groups, are defined by the existence of a carbon-carbon double bond (C=C) inside a molecule. A peak in the wavenumber region of 1680-1630 cm^{-1} is the primary indication for olefins. The stretching vibration of the carbon-carbon double bond is correlated with this peak (C=C) [14, 25-27].

Generally, unconjugated alkenes (isolated double bonds) show a medium-strength peak. Conjugated alkenes (double bonds with alternating single and double bonds) can have a weaker and broader peak due to delocalization of electrons. While the C=C stretch is the most prominent indicator, other peaks might be present depending on the specific molecule. Additionally, out-of-plane bending vibrations for the olefin hydrogens can be observed in the 1000-650 cm^{-1} region [26, 28, 29]. The FTIR analysis spectra presented in Fig. 5 also indicate the presence of phenols, a group of aromatic compounds that have one or more hydroxy groups directly linked to the benzene ring [15, 23]. The characteristic peak for alcohols is strong in the range of 1260-1050 cm^{-1} [15]. This peak arises from the stretching vibration of the C-O bond (primary, secondary, tertiary) [18, 20]. Similar to alcohols, phenols possess aromatic rings with characteristic peaks in the 1600-1400 cm^{-1} region. These peaks relate to various stretching vibrations within the aromatic ring. Phenols also show a C-O stretching vibration peak in the range of 1260-1200 cm^{-1}, similar to alcohols.

3.4. Rheological analysis

The rheology test result of CRO is presented in Fig. 6 (A) and (B) showing the results of a rheological test performed on a CRO sample. The test measures the viscosity of the sample at different temperatures. Fig. 6 (A) shows the viscosity of the sample as a function of temperature. The viscosity appears to decrease as the temperature increases. This indicates that when the shear rate increases, the sample's viscosity will decrease.

The sample's storage modulus (G') and loss modulus (G") as a function of temperature appear to be displayed in Fig. 6 (B). The elastic characteristics of the sample were measured by the storage modulus, and its viscous characteristics are measured by the loss modulus. The storage modulus appears to be larger than the loss modulus at all temperatures, which means that the sample is more elastic than viscous. Overall, the results of the rheological test suggest that the sample is a shear thinning fluid that is more elastic than viscous.

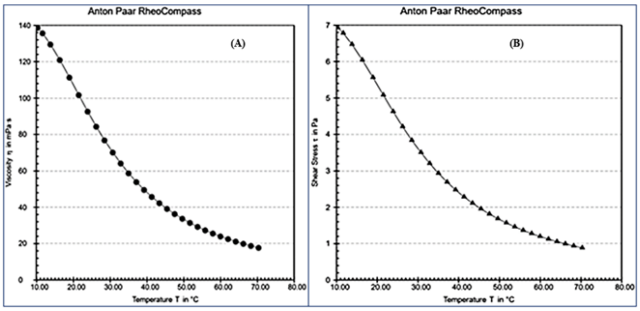

Fig. 5. Rheological analysis results illustrating (A) viscosity and (B) sheer of CRO extracted from medium particle size of Birbira seed powder by Soxhlet extraction method with chloroform solvent.

4. Conclusions

The main purpose of this research work was to investigate of the best extraction method and evaluation of the optimum parameters leading to highest percentage of CRO yield from Birbira plant seed. As a result, the two extraction methods, Soxhlet and maceration extraction methods were performed applying three different solvents (ethanol, hexane and chloroform) on three different Birbira seed powder particle sizes (fine, medium and coarse). It was observed that the Soxhlet extraction method is more efficient than the maceration method. In addition, to the extraction method, Birbira seed powder particle sizes and solvent types were evaluated and medium particle size (0.10-0.35mm) was investigated to be the optimum seed powder particle size and chloroform was the best solvent compared to ethanol and hexane. Therefore, the extraction of CRO from Birbira plant seed was optimized in terms of extraction method, Birbira seed powder particle size and solvent type as Soxhlet extraction method, medium particle size and chloroform solvent respectively. The crude rotenone oil was characterized by using FTIR analyser and Rheocompass equipment. The FTIR result revealed that crude rotenone oil is composed of aliphatic ester, olefin, alcohol and phenol functional groups.

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