MALAYSIA

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TITLE	:	SWIETENIA MAHAGONI SEEDS OIL AND METHOD OF EXTRACTION
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SWIETENIA MAHAGONI SEEDS OIL AND METHOD OF EXTRACTION

Field of Invention

The present invention relates to antibacterial properties of Swietenia mahagoni seed oil and to a method of extraction of *Swietenia mahagoni* seeds oil.

BACKGROUND OF THE INVENTION

Swietenia mahagoni Jacq. (Meliaceae), grows mainly in tropical areas of Asia, such as India, Malaysia, Indonesia and southern mainland China. Its seeds have been applied as folk medicine for treatment of hypertension, diabetes, and malaria, while the decoction of its bark has been used as a febrifuge (Chen, *et al.* 2007). The therapeutic effects associated with the seeds are mainly caused by the biologically active ingredients, fatty acids and tetranortriterpenoids (Bascal, *et al* 1997). There are reports of *S. mahagoni* seeds having anti-inflammatory, antimutagenecity, and antitumour activities (Guevara *et al.*, 1996). The plant extracts have been accounted to possess antibacterial and antifungal activities. Limnoid obtained from *S.mahagoni* have antifungal activity and diabetes therapy (Alrdahe, *et al.*2010). The seeds of *S. mahagoni* are good agriculture product and have been found potentially rich in fat (64,9%) (Ali, *et al.* 2011).

The traditional procedures for the extraction of plant materials include hydrodistillation and organic solvent extraction using percolation, maceration or soxhlet techniques. However, there are drawbacks with these methods such as time and labour consuming operation, and involves large volumes of hazardous solvents. Nevertheless, there is increasing interest in alternative extraction methods that consume smaller quantities of organic solvent due to the rising solvent acquisition and disposal costs and regulatory restrictions (Khajeh, 2011). Therefore, it is highly desired to develop alternative extraction techniques with better selectivity and efficiency. Consequently, Supercritical fluid extraction (SFE) as in environmentally responsible and efficient extraction technique for solid materials was introduced and extensively studied for separation of active compounds from herbs and other plants (Lang *et al.*2001).

MA. Majid et.al disclosed that oil extracted from Swietenia mahagoni seed was studied with a view to finding out its suitability for ethnomedical uses with special focus on antimicrobial and toxic behavior. Some of its physical and chemical properties were examined and compared with those of standard oils: olive, sunflower, cotton seed, Linseed, soybean, coconut, palm and castor. The refined oil was found to show good to moderate activity against disease causing bacteria viz. Shigella dysenterial, Salmonella typhi, Staphylococcuss aureus and fungal pathogens viz. Macrophomina phascolma, Alternaria alternata, Curvularia lunata. Different extracts from Swietenia mahagoni seed showed minimal toxic effect while applied on predatory fish's viz. Heteropneustes fossilis and Anabas testudineus.

Sahgal et.al disclosed the present study was designed to evaluate the antibacterial activities of Swietenia mahagoni crude methanolic (SMCM) seed extract. The antimicrobial activity of the oily extract against Gram-positive, Gram-negative, yeast and fungus strains was evaluated based on the inhibition zone using disc diffusion assay, minimal inhibition concentration (MIC) and minimal bactericidal concentration (MBC) values. The crude extract was subjected to various phytochemicals analysis. The demonstrated qualitative phytochemical tests exhibited the presences of common phytocompounds including alkaloids, terpenoids, antraquinones, cardiac glycosides, saponins, and volatile oils as major active constituents. The SMCM seed extract had inhibitory effects on the growth of Candida albicans, Staphylococcus aureus, Pseudomonas aeroginosa, Streptococcus faecalis and Proteus mirabillase and illustrated MIC and MBC values ranging from 25 mg/ml to 50 mg/ml.

PI2010003170 disclosed a process for extraction standardised antioxidant and catechin extracts from *Morinda citrifolia* leaf utilizing a supercritical fluid extraction (SFE), the process includes the steps of (a)pretreating a sample of *Morinda citrifolia* leaf, (b) conducting a SFE using carbon dioxide and polar modifier for a period of approximately one to five hours, preferably three hours and collecting ethanol containing solute. The extractions are performed at 40°C to 60°C, 10 to 30 MPa and carbon dioxide flow rate at 15 to 25g/min.

Lang and Wai (2001) have reviewed several advantages of SFE which are: (1) Shorter extraction time when using supercritical fluids, since they have relatively lower viscosity and

higher diffusivity.(2) Continuous fluid flowing though samples could provide quantitative or complete extraction, (3) SFE is usually performed at low temperature, (4) The solvent power of the fluid can be adjusted by varying the pressure and temperature, (5) Separation of solutes dissolved in supercritical fluid can be easily done by depressurization, (6) Only a little quantity of sample is needed, (7) Zero or little amount of organic solvent is needed, which is environmentally responsible.

SUMMARY OF THE INVENTION

An aspect of the present invention to provide a method for extraction of seed oil of Swietenia mahagoni comprising the step of:

a. providing ground Swietenia mahagoni seed;

b. subjecting the ground Swietenia mahagoni seed to a Supercritical Carbon Dioxide extraction system;

c. obtaining oil extract of Switenia mahagoni seed;

characterized in that; step (b) is conducted at carbon dioxide flowrate range from 2 to 4 ml/min, at pressure ranges from 20 to 30 MPa; and temperature ranges from 40 to 60°C.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1: illustrates antimicrobial activity of the extracts on *Bacillus subtilis* (A), *E. coli* (B), *S. aureus* (C) and *P.aeroginosa* (D): Disc diffusion test for the effect of *S.mahagoni* grown on nutrient agar medium.1.100% Methanol; 2. 70% methanol; 3.100% Ethanol; 4. 70% Ethanol; 5.Aceton; 6.Negative control.

Figure 2: illustrates a schematic design of the supercritical fluid extraction (SFE) unit.

Figure 3. illustrates percentage of extraction yield on different Flow rate CO_2.

Figure 4. illustrates DPPH free radicals scavenging activity of *Swietenia mahagoni* extracts at different of Flow rate CO_2.

Figure 5. illustrates total phenolic content of *Swietenia mahagoni* extracts at different of flow rate CO_2.

DETAILED DESCRIPTION OF THE PRESENT INVENTION

The present invention to provide antibacterial composition comprising effective amount of oil extract *of Swietenia Mahagoni* seed.

Additionally, the antibacterial composition is effective against broad spectrum of pathogenic bacteria which includes *Bacillus subtilis, Staphylococcus aureus, Escherchia coli* or *Pseudomonas aeruinosa* as illustrated in Figure 1.

Advatageously, the oil extract comprises a phenolic compound. One known phenolic compound is gallic acid.

Generally, a method for extraction of seed oil of *Swietenia mahagoni* comprising the step of: a.providing ground *Swietenia mahagoni* seed; b.subjecting the ground *Swietenia mahagoni* seed to a Supercritical Carbon Dioxide extraction system; and c.obtaining oil extract of *Switenia mahagoni* seed.

Preferably, the step (b) is conducted at pressure ranges from 20 to 30 MPa.
Preferably, the step (b) is conducted at temperature ranges from 40 to 60^{o}C.
Preferably the step (b) is conducted at carbon dioxide flowrate ranges from 2-4ml/min.

Generally, the method of extraction of *S. mahagoni* seed oil is by using Supercritical Fluid Extraction of the present invention. Supercritical fluid extraction (SFE) as in environmentally responsible and efficient extraction technique for solid materials was introduced and extensively

studied for separation of active compounds from herbs and other plants. Several advantages of SFE are; supercritical fluids have relatively lower viscosity and higher diffusivity, which results in shorter extraction time, continuous fluid flowing through samples could provide quantitative or complete extraction, SFE is usually performed at low temperature, the solvent power of the fluid can be manipulated by changing pressure and temperature, solutes dissolved in supercritical fluid can be easily separated by depressurization, little sample quantity is needed, little or no organic solvent is need, which is good for the environment.

Extraction preparation using Soxhlet apparatus was conducted as comparison and to show that seed oil extract of *Swietenia mahagoni* exhibit antibacterial properties. Extraction using SFE is environmental friendly and safer to consume compared to using Soxhlet apparatus.

Antibacterial composition of the present invention comprising of *S. mahagoni* seed oil, the *S.mahagoni* seed oil is usable in the preparation of soaps, body ointments and hair oil. The seeds' oil is the same used in many handmade, detergent-free bath bars sold by spas. Oils of palmitic and oleic acids, which make up 85 percent of forest *Swietenia mahagoni* seeds' oil content, make vegan-friendly soaps. Palmitic and oleic acids are also key ingredients in hair oils. The emollient properties of *S.mahagoni* seed oil improve the texture of hair as well as detangle it.

The oil produced from *Swietenia mahagoni* seeds' palmitic and oleic acids can serve as an alternative medicine therapy to treat atopic dermatitis, or eczema. *Swietenia mahagoni* seed oil has been used as an alternative body ointment therapy for a range of skin cuts, itches and wounds to ameliorate the healing process.

Swietenia mahagoni seeds contain 33 types of flavonoids and 27 types of saponins, making them even more nutritionally rich those other nutrient-dense plants, such as their relative ginseng. They also contain various vitamins, minerals, proteins and fatty acids that are necessary to healthy body function.

One of the greatest advantages of *Swietenia mahagoni* seed is its effect on improving blood circulation. The flavonoids in *Swietenia mahagoni* seed directly aid in improving circulation, while the saponins are good for heart health. This combination of nutrients in *the Swietenia mahagoni* seed makes it a good natural treatment to encourage good heart health and better circulation.

The present invention will be described in more detail below with reference to the following examples, but the present invention is not restricted to these specific examples at all.

Example 1

MATERIALS AND METHODS

Plant Materials

The seeds of *S.mahagoni* used in this study will be obtained from Indonesia. Then, the seeds will be washed under tap water in order to remove undesired particle. After that, all of the seeds will be dried using oven at 50 °C. The seeds were powdered using a blender.

Extraction Preparation using Soxhlet apparatus

The powdered seeds of *S.mahagoni* (15 g) were extracted with 100 % methanol using Soxhlet apparatus. Lastly, the extraction yield will be put in rotary evaporator at 40°C to remove the solvent. All the step will be repeated using 100% ethanol, 70% methanol, 70% ethanol, acetone. The concentrated extract was dried in oven at 50° for three days to obtain consistent weight. The sample was stored under refrigeration (-20°C) condition for further analysis.

Antimicrobial assay

S.mahagoni methanolic, ethanolic, acetone seed extract will be subjected to antimicrobial assays including agar disc diffusion test, and broth dilution assay.

Microbial strains

Four pathogenic microorganisms namely *Escherchia coli, Staphylococcus aureus, Bacillus subtilis* and *Pseudomonas aeroginosa* culture were used for antimicrobial assays . The bacteria strains were grown in 50 ml of nutrient broth at 37°C and maintained in nutrient agar slant at 4°C.

Agar disc diffusion assay

The agar diffusion method (Murray, et al.1995) was used to evaluate the antimicrobial activity of the subjected extracts. Inoculum of 100 µl suspension containing 10^8 CFU/ml of bacteria were spread on Mueller Hinton Agar. The discs (9 mm in diameter) impregnated with 20 µl of 100 mg/ml extracts were placed on seeded agar medium. Streptomycin (10µg/disc) were used as positive control for bacteria. Methanol was used as negative control. The experiments were conducted in triplicate and the test plates were incubated 24 hours at 37º C for bacteria. The diameters of zone of inhibition measured in mm (Mandal,et al.,2000)

RESULT AND DISCUSSION

The result of disc diffusion assay of methanol, ethanol, and acetone extract of the seed of *Swietenia mahagoni* have been tabulated in Table-1 and Figure-1. It is evident from Table-1 that methanol, ethanol, and acetone extract was found to be active againtas the bacteria like *Escherchia coli, Staphylococcus aureus, Bacillus subtilis* and *Pseudomonas aeruginosa*. The results of disc diffusion assay of the crude extract were compared with that standart antibiotic Streptomycin (10μg/disc). Results also proved that methanol extract has more effectiveness than that of ethanol and aceton extract against subjected bacteria strains. The gram-negative bacteria *Pseudomonas aeroginosa* were more sensitive to methanol, ethanol, and aceton extracts.

Besides the seed, other parts of this plant such as leaf and bark have also been tested for antimicrobial activities by using chloroform and ethyl acetate by Haque *et al.* (2009). Several limnoids were previously isolated from S.mahagoni (Govindachari *et al.*, 1999). Isolation of triterpenes also was reported on *S.mahagoni* (Ekimoto *et al.*, 1991). The antibacterial activity of the isolated limnoids 1 and 2 has been reported by Shahidur rahman *et al.*, (2009) against pathogenic bacteria. Phytochemical and antimicrobial activity of *Swietenia mahagoni* crude methanolic seed extract (Sahgal, et al.,2009).

From above results it can be concluded that the seeds of plant *Swietenia mahagoni* possess significant antimicrobial activity in term of antibacterial. This antimicrobial property against bacteria to presence of some antimicrobial substances in seed.

Table 1. Antibacterial activity of the extract by disc diffusion assay

Name of the solvent extract and antibiotics	*Inhibition zone (mm)*				
	Extract yield (%)	*Bacillus subtilis*	*Staphylococcus aureus*	*Escherchia coli*	*Pseudomonas aeruinosa*
100 % Methanol	*25.64*	*11.00*	*11.00*	*10.00*	*14.00*
70 % Methanol	*11.86*	*13.00*	*10.00*	*07.00*	*13.00*
100 % Ethanol	*40.83*	*07.00*	*10.00*	*07.00*	*12.00*
70 % Ethanol	*19.27*	*12.00*	*11.00*	*11.00*	*13.00*
Aceton	*34.24*	*12.00*	*11.00*	*08.00*	*14.00*
Streptomycin	-	*18.00*	*17.00*	*16.00*	*14.00*

Example 2

Method of extraction using Supercritical Carbon Dioxide (SC-CO_2)

The system of supercritical carbon dioxide extraction consists of: high-pressure pump, 50 ml extraction vessel, automated back pressure regulator, oven and liquid CO_2 is supplied from a gas cylinder. A schematic design of the SC-CO_2 unit used in this work is shown in Figure.1 Extraction is conducted under three independent variables namely pressures (20 – 30 MPa), temperature (40 – 60°C) and CO_2 flow rate (2 – 4 ml/min) and the extraction is done for 120 minutes. 5 g of ground S.mahagoni seed was placed into the extraction vessel. Cotton was placed at the end to avoid any possible residue of solid material. The vessel was placed in an oven to maintain operating temperature. The extraction yield was collected in vial and placed in an oven to allow for evaporation solvent. And then, the extract was weighted and calculation of concentration yield is done based on cumulative mass of extract.

Result

Percentage of Extraction Yield by SC-CO_2

Figure 2 shows the result of yield on *Swietenia mahagoni* seed extract. There are three different flow rate CO_2 were used to extract the *Swietenia mahagoni* which are 2 ml/min, 3 ml/min and 4 ml/min.

The curves shows that the yield increases with an increase in the supercritical carbon dioxide flow rate, reached a maximum value, and then decreases with further increases in the flow rate. Based on the obtained results, the highest extraction yield was found with 4 ml/min flow rate CO_2 and then followed by 3 ml/min. On the other hand, 2 ml/min flow rate CO_2 resulted in lowest extraction yield.

DPPH Free Radicals Scavenging

Figure 3. shows the DPPH radical scavenging assay result of three different flow rate carbon dioxide. The flow rate CO_2 2 ml/min (94.40%) gives highest DPPH radical scavenging activity compared to 3 ml/min (91.13%) and 4 ml/min (90.37). This indicates the antioxidant ability of flow rate carbon dioxide 2 ml/min oils is stronger than the other two oils.

Total Phenolic Content

Total phenolic content, as determined by the Folin Ciocalteu method, is reported as gallic acid equivalents by reference to standard curve ($y = 0.00064x - 0.03186$) and $r^2 = 0.986$). The total phenolic content extract is shown in Figure 4.

The flow rate CO_2 3 ml/min have higher phenolic content (102 mg/g sample) compared to other (2 ml/min 66 mg/g sample and 4 ml/min 62 mg/ml sample). That may be responsible for the antioxidative activities of this extract.

Antibacterial Activity

The antibacterial activity of Swietenia mahagoni seed extract using different solvents was studied by using disc diffusion method and the results are tabulated in Table 2. All the samples were tested again one gram negative bacteria (*Escherchia coli*) and two gram positive bacteria (*Bacillus subtilis* and *Stapylococcus aureus*). The inihibition zone was measured after 24 hours.

Table 2: Antibacterial activity of different extracts of *Swietenia mahagoni* seed extract at 24 hours

Extract of CO_2 Flow rate	*E.coli*	*B.subtilis*	*S.aureus*
2 ml/min	9.5 ±0.71	8.5 ± 2.12	9.5 ± 0.71
3 ml/min	13.5 ± 2.12	12.0 ± 1.41	11.5 ± 0.71
4 ml/min	12.5 ± 2.12	9.0 ±2.83	12.5 ±2.12
Positive control	15.5 ± 0.71	15.0 ± 0.00	13.0 ± 1.41

While the preferred embodiment of the present invention has been describe, it should be understood that various changes, adaptation and modification may be made thereto. It should be understood, therefore, that the invention is not limited to details of the illustrated invention shown in the figure and that variation in such minor details will be apparent to a person skilled in the art.

CLAIM

1. A method for extraction of seed oil of *Swietenia mahagoni* comprising the step of:
    a. providing ground *Swietenia mahagoni* seed;
    b. subjecting the ground *Swietenia mahagoni* seed to a Supercritical Carbon Dioxide extraction system;
    c. obtaining oil extract of *Switenia mahagoni* seed;

    characterized in that

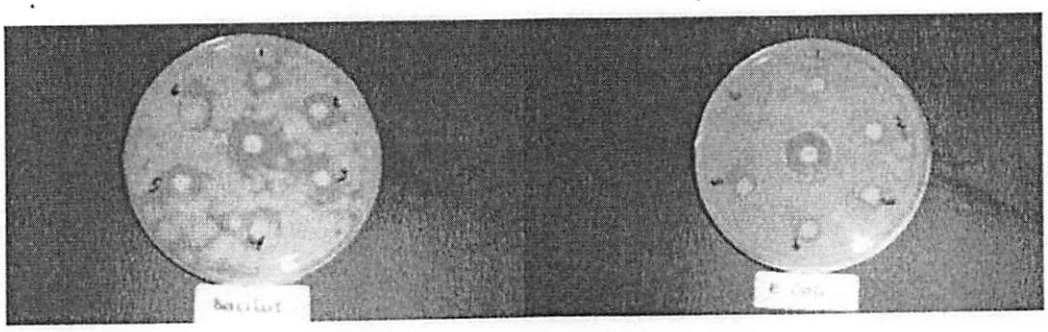
    the step (b) is conducted at carbon dioxide flowrate ranges from 2 to 4 ml/min;
    the step (b) is conducted at pressure ranges from 20 to 30 MPa; and
    the step (b) is conducted at temperature ranges from 40 to 60^{o}C.

ABSTRACT

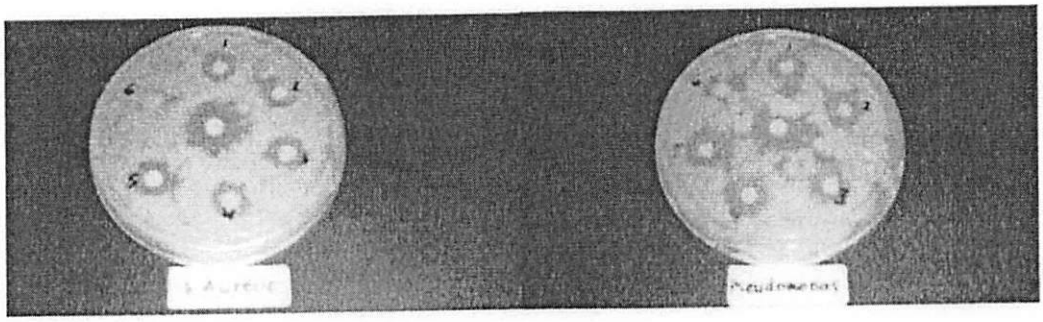
SWIETENIA MAHAGONI SEEDS OIL AND METHOD OF EXTRACTION

The present invention relates to a method for extraction of *Swietenia mahagoni* seeds oil. The method comprises the steps of;providing ground *Swietenia mahagoni* seed; subjecting the ground *Swietenia mahagoni* seed to a Supercritical Carbon Dioxide extraction system; and obtaining oil extract of *Switenia mahagoni* seed.

Most illustrative Figure: **FIGURE 3**



(A) (B)



(C) (D)

FIGURE 1

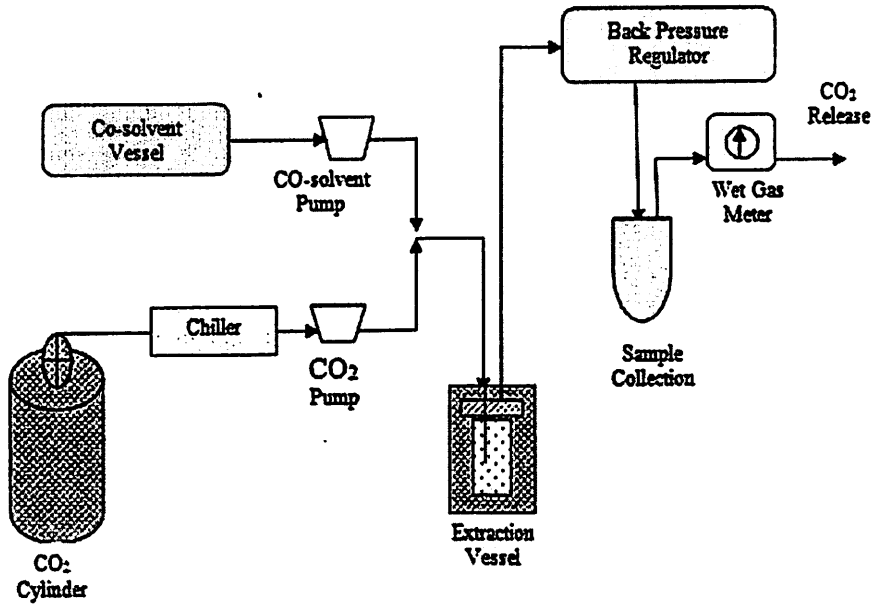


FIGURE 2

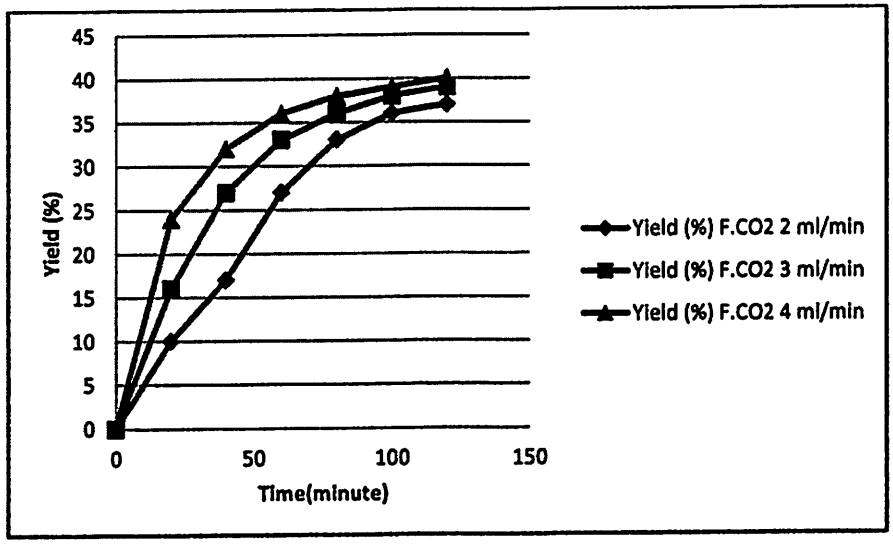


FIGURE 3

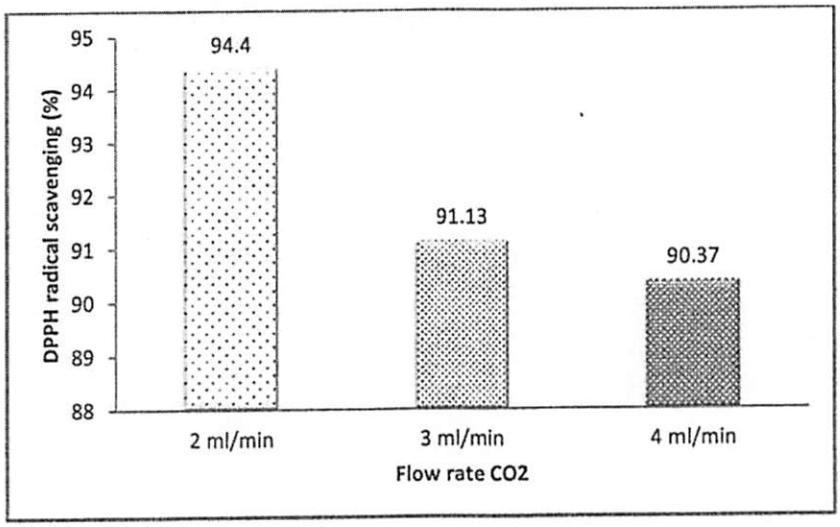


FIGURE 4

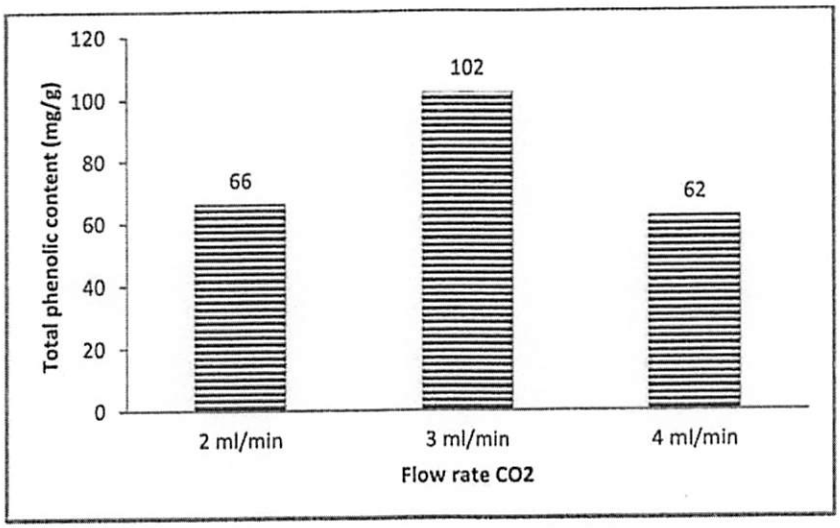


FIGURE 5