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Extraction And Identification of Essential Oil from The Flower Artabotrys Hexapetalus for Its Volatile Compounds

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Abstract

Essential oils are hydrophobic compounds which contain volatile compounds. Essential oils contain either essential amino acids or fatty acids. Here essential oil is extracted from *Artabotrys hexapetalus* flowers. Initially the flowers of this plant undergo steam distillation process. The oil extracted is centrifuged and so pellet in the form of crystals is formed. The formed pellet is dried and are used for further analysis. The analysis tells us about the presence of amino acids, flavonoids, etc., found in essential oil. The formed pellet was subjected to FTIR analysis for validation and purity of the oil. The free radical scavenging activity was performed for the oil sample. DPPH (2,2-diphenyl-1-picrylhydrazyl) is used to assess the essential oil sample from the taken flower. The sample is also subjected under the analyzation of UV/Vis spectrophotometric instrumentation. To analyze the chemical compounds, present in the essential oil, Gas Chromatography mass spectroscopy (GC-MS) was also performed. This analysis found the essential components of an essential oil which makes it fragrance and acting against microbes. This oil also identified to have larvicidal activity against mosquito. Anti-microbial activity of essential oil is observed for the effective application of this Essential Oil in the pharmaceutical environment and also to enhance its role in aromatherapy.

Key words: *Artabotrys hexapetalus*, Essential Oil, Antioxidant Activity, Larvicidal activity, Anti-microbial activity.

Introduction: Essential oil is a characteristic compound found in plant sources. These are the compounds present in various kinds of the plant particularly in blossoms with a profoundly sweet-smelling smell thus, it is known as essential oil. The presence of aliphatic and phenolic intensifies makes it sweet-smelling and furthermore have the properties of hostile to parasitic, against bacterial and hostile to oxidant. These EO's are intensifies which are distinguished not having any impacts conceivably when utilized as substitute as seasoning for nourishments [27]. There are around 3000 EO's available in which 300 oils are monetarily significant [2]. Essential oils also have many nutritional values which have many benefits for human beings. Essential oils are now-a-days considered as alternative option and believes to be the complementary therapy for the treatment of many diseases [16]. Essential oils likewise have numerous healthy benefits which have numerous advantages for people. In this investigation, *Artabotrys hexapetalus* blossom is utilized to acquire the essential oil. This plant has a place with the family 'Annonaceae' and the class Artabotrys [18]. It is a grand evergreen tree, generally planted because of its adequacy in lightening commotion contamination. The tree is known to develop more than 9 m in stature. Concentrates also, exacerbates segregated from the plant have been accounted for to have various natural exercises [26]. The common name of this *Artabortys hexapetalus* is ylang ylang flower and its Tamil name is 'manoranjitham'. This ylang ylang flower spreads pleasant fragrance in an evening and mostly all flowers of ylang ylang arises from stem which seems to be in the hook-like structure. The decoction of the flowers is used for tea and leaves extraction is used to treat cholera [10]. The Artabotrys genus have around 101 to 110 species. Still, only 12 species of Artabotrys undergone a study for the extraction of essential oil [29]. Using hydro-distillation method, essential oil from flower is going to be performed. Meanwhile, the research is also done in stem and root and so, for the novelty and new outcome the ylang ylang flower of annonaceae family was chosen. The anti-oxidant property ;of the oil and larvicidal activity against mosquito is also performed to increase the economic importance of the plant. To find the chemical compounds present in the essential oil, Gas chromatography and mass spectroscopy is performed in this study [8,17].

Materials and methods: The ylang ylang *(Artabotrys hexapetalus)* flower was collected from the nearest market. It is verified with research sources for the right chosen of sample. Totally, 50 g of sample were taken for the extraction of essential oil.

Extraction of essential oil: Initially, 50 g of flower sample was taken. The sample was washed with distilled water for two times. In the 250 mL round bottom flask, 200 mL of distilled water was taken with which the 50 g of flower was added. The set up for steam distillation was done using proper apparatus as mention in the previous papers. For two hours, the distillation process proceeded. After the end of 2 hours, 25 mL of essential oil was collected in the form of milky white layer miscible with water. It has a good smell of the flower.

Crystal formation of oil: The essential oil was obtained in the form of water solution. It was then converted into crystal format using a technique "centrifugation". The centrifugation of the oil was performed using the centrifuge instrumentation. At 14 ℃, the centrifugation was done under 10,000 rpm for 10 minutes. The pellet and supernatant were obtained separately. The supernatant was discarded meanwhile, the pellet was collected. The collected pellet was dried in a hot air oven at 45 ℃ until, the pellet gets completely dried and turned as crystals.

UV-spectrophotometer: The chemical composition of the EO of *Artabotrys hexapetalus was* analyzed by using UV spectrophotometer. The essential oil obtained from steam distillation which was in the solution form subjected to the analysis. In this analysis, ultra-violet – visible absorption principle is used. These focusses, the molecules have bonding and non-bonding electrons which absorbs the Ultra-violet or visible as energy for getting excited to higher anti – bonding molecular orbitals. Based on the Beer – Lamberts law assumption the obtained results were quantified. The whole 25 mL procured essential oil from the *Artabotrys hexapetalus* flower of 50 g was subjected to the UV – Vis spectroscopic analysis. The absorbance curve was taken from 200 nm to 700 nm [9].

Anti-oxidant assay: The free radical scavenging property which is commonly known as antioxidant property was done to the solution form essential oil [21]. According to the procedure reported by Dildar Ahmed *et al.,* which is the slight modification method of Brand-Williams *et al* the assay was performed. Here, the DPPH free radical scavenging method was performed. Initially, 0.024 g of DPPH chemical was taken and that was mixed with 100mL of methanol. This primarily prepared solution was considered as stock solution. The stock solution was stored in refrigerator until it had been used. Then, the working solution was prepared from the previously made stock solution by diluting it in a methanol until the absorbance value reached of about 0.98 (±0.02) at 517 nm in UV/Vis spectrophotometer.

In a clean test tube, 3 mL of working solution was taken. In that 100 µL of test sample which is essential oil was added. After a proper mixing, an absorbance value was taken at 517 nm especially for a period of 30 minutes of time. The free radical scavenging property was calculated using following formula,

$$\% \text{ Anti-oxidant activity} = [(Ac - As)/Ac] \times 100$$

Here,

Ac – control absorbance

As – sample absorbance (EO)

For making a control solution, test sample of 100 µL was replaced by 100 µL of methanol. Like that 200, 400, 600 and 800 µL of essential oil was analyzed for the absorbance value [1].

Anti - microbial activity: The anti-microbial activity of EO is the most important thing which make it a desired one. The anti-microbial activity is nothing but the activity which act against microbes [19]. In this study, the anti-microbial activity was checked using the technique call well diffusion method [12]. The nutrient agar medium containing plates were done before done. The plates were made ready by

autoclaving the plate at 121℃ for 15 minutes under 15 psi. The plates were incubated for overnight in incubation chamber for the proper sterility check of the plate. The water sample from running tap water was taken. Then, the water was serial diluted from 10^{-1} to 10^{-9} concentration. After the sterility check, the plates were made ready for inoculation of sample. From the serial dilution, concentration of 10^{-7} to 10^{-9} were taken and spread plate technique was performed. After the spread plating the well was punctured carefully in an agar. The four different concentrations of essential oil like 10 µL, 20 µL, 30 µL and 40 µL was tested. The plates were kept undisturbed for 24 hours at 35 ℃. After that the zone of inhibition of the plates were measured [23].

GC-MS analysis: Gas chromatography-Mass spectroscopy is an instrument utilized to identify the chemical compounds which is present in the sample. The ylang-ylang flower essential oil which is made into crystals was given for the GC-MS analysis. It was given to chemistry division for NMR and GC-MS analysis spotted in VIT-SIF Laboratory, SAS. The GC of Clarus 680 was used which utilized a column of fused silica. The Elite-5MS (5 % biphenyl 95 % dimethylpolysiloxane, 30 m × 0.25 mm ID × 250 µm df) was utilized for packing the column and the helium gas was utilized as gas of carrier in constant flow as 1 mL/min. On the time of chromatographic run, the temperature of injector was set at 260 °C. During the sample injection, the temperature of oven was controlled at 60 °C (2 min); followed by 300 °C at the rate of 10 °C min−1; and 300 °C which was held for 6 min. The conditions of Mass detector were including, temperature of transfer line 230 °C; temperature of ion source 230 °C. The electron force of ionization mode was set at 70 eV, with a scan time of 0.2 sec and 0.1 sec scan interval. The obtained components spectrums were correlated with the spectrum database of known chemical components which was stored in the NIST (2008) library of GC-MS.

Mosquito Larvicidal activity assay: Mosquitos are the most dangerous and also known as vector for many series diseases like malaria, dengue, yellow fever and some more diseases through their bites. To stay away from that, more precautions are identified. To be a part of that, the obtained essential oil of the ylang ylang flower was tested for the larvicidal activity against larva of mosquito. The larvae of mosquito were collected gently from stagnant pool. According to the protocol mentioned in research paper [5], the larvicidal activity was performed. The collected mosquito larvae were properly maintained and fed for some time before performing the assay. In this study, essential oil of flower was taken in 250 mL beaker in 100 mL in which 150 mL of distilled water which contained 0.1 % tween 80 was added. Then, the maintained larvae were incubated in the prepared solution of essential. The control was also prepared with distilled water with the same proportion of tween 80 without adding essential oil. To this also, larvae were incubated.

Results and discussion:

UV/Vis spectrophotometer:

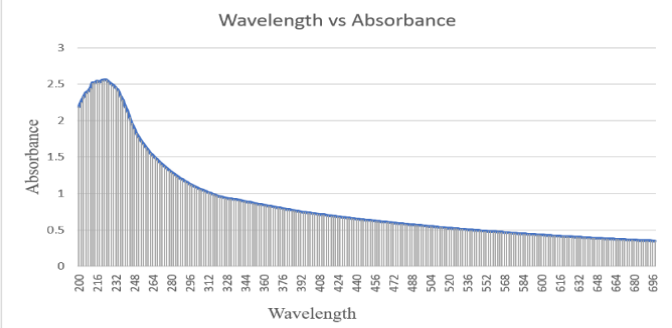

Figure 1: Graph between Wavelength vs absorbance

S.No	Concentration of sample(ml/ml)	Absorbance at 517nm	% Anti-oxidant activity
1.	0.2	0.661	33.23 %
2.	0.4	0.615	37.87 %
3.	0.6	0.589	40.5 %
4.	0.8	0.543	45.15 %
5.	1.0	0.401	59.49 %

The UV/Vis spectrometric analysis was performed to find out the major compound present in the essential oil of *Artabotrys hexapetalus* flower. The scan parameter was chosen and the whole sample was analyzed from 200 nm – 700 nm. The graph was obtained and it shows the peak at wavelength of 222 nm with the absorbance of 2.562. This shows that essential oil contains strong peptide bonds which is formed by amino acids. Peptide bond is the covalent bond formed between two amino acids. It is also been basis for many biological reactions. For the peptide bond, bond edges and bond lengths show that carbon–nitrogen bonds have a lot of twofold bond character, and that the C, O, N, and H atoms all lie in a similar plane. The ϕ and ψ allude to revolutions about the single bonds associating the α-carbon with the α-nitrogen and the α-carbonyl carbon, separately. It indicates essential oil contains various types of amino acids which codes for the different proteins [4]. These confirms the presence of different functional groups in the EO of *Artabotrys hexapetalus.*

Anti-oxidant assay:

Table:1 Result of anti-oxidant test

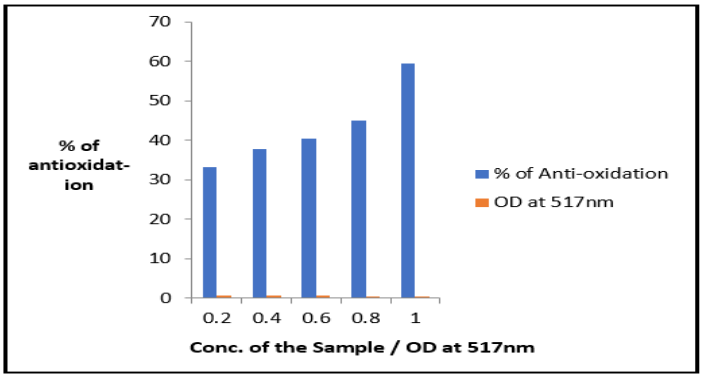

Figure 2: Graph between % of anti-oxidant property vs conc. Of the sample

This was the test done for determining the free radical scavenging activity. In all human systems, there is a compound called reactive oxygen which is a highly unstable one. These compounds are produced as the intermediate in many enzymatic reactions. These may tissue damage and ageing process in humans. Even sometimes, feeling tired and sleepy during day time may due to this reactive oxygen species. To overcome this coffee and tea have been drunken by the humans which supports rejuvenation of tissues [3]. Tran D. These *Artabotrys hexapetalus* flowers from an Indian sub-continent being used as tea making and also used to treat cholera. This study also climbs the similar thing that these EO has a free radical scavenging activity. It was determined by using DPPH assay [7]. This is also plotted in the form of graph for the undemanding comprehension. From the graph, the antioxidant property of the essential has been observed. The five different concentrations which was taken for the

test sample was 0.2 ml, 0.4 ml, 0.6 ml, 0.8 ml and 1ml. These concentrations were tested according to the procedure which was tabulated for the understanding. The percentage of anti-oxidant property of an EO was find out using the absorbance formula. The activity of free radical scavenging was increased from low concentration to the high concentration of essential oil [6].

Anti-microbial test: This test effectively confirms the anti-microbial activity of essential oil of *Artabotrys hexapetalus* flower. The zone of inhibition was visualized from an incubated plate [14]. The anti-microbial activity of this EO is due to the presence of many chemical groups namely β-caryophyllene, caryophyllene oxide, ylanga-2,4(15)-diene etc. According to the research of Giang M. Phan *et al*., the essential of this *Artabotrys hexapetalus* flower contains totally twenty-six compounds which includes sesquiterpene hydrocarbons (33.3 % of EO) and oxygenated sesquiterpenoids (47.7 %).

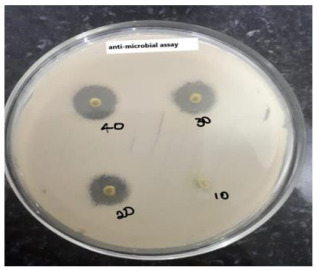

Figure 3: Anti-microbial test

These many compounds being cause for the anti-microbial activity. Commonly these mentioned compounds are found to have anti-inflammatory activity and so that, it is being used as a non – steroidal anti-inflammatory drug [25]. These compounds also found reason behind the fragrance of essential oil and as well as the fragrance of flower. In common, those compounds are metabolites found in all over the plant.

GC-MS analysis:

	Compound name	**Molecular weight**	**Chemical formula**
1.	Ethyl acetate	88	$C_4H_8O_2$
2.	4-Hydroxy-2-Butanone	88	$C_4H_8O_2$
3.	4-(Acetyloxy)-2-butanone	130	$C_8H_{10}O_3$
4.	2-Butyne-1,4-diol, diacetate	170	$C_8H_{10}O4_4$
5.	Cis-2-chlorovinylacetate	120	$C_4H_5O_2$
6.	Trans-traumatic acid	228	$C_{12}H_{20}O_4$
7.	1-monolinoleoylglycerol trimethylsilyl ether	498	$C_{27}H_{54}O_4Si_2$
8.	2,6-lutidine 3,5-dichloro-4-dodecylthio-	375	$C_{19}H_{31}NCl_2S$
9.	1,3-Bis-t-butylperoxy-phthalan	296	$C_{16}H_{24}O_5$
10.	2-(Aminooxy) pentanoic acid	133	$C_5H_{11}O_3N$
11.	Pseduosarsasapogenin-5,20-dien methyl ether	428	$C_{28}H_{44}O_3$
12.	1,4-cyclohexadiene, 1,3,6-tris(trimethylsilyl)-	296	$C_{15}H_{32}Si_3$

Table 2: Result Of GC-MS analysis

Ethyl acetate was found to be the most abundant component in essential oil of ylang ylang flower. The second and third most abundant component was found to be pentanoic and traumatic acid respectively. Other than that, around ten components were identified in the essential oil. The compound 1 which was ethyl acetate is an ester form of ethanol and acetic acid and also possess the sweet, fruity odor. This compound may be cause for the odor of ylang-ylang flower essential oil. 4-Hydroxy-2-butanone and 4-(Acetyloxy)-2-butanone was an identified compound which gives several chemical compounds under specified condition. 2-Butyne-1,4-diol was broadly adopted for the cycloaddition reaction which is the homologation method for substituted acenes preparation. It was also utilized in the synthesis of bistramide A and amphidinolide P. Trans – traumatic acid was a chemical compound which has the wound healing property which is induced by the diabetes mellitus. 2-(Aminooxy) pentanoic acid compound present in the essential oil of ylang ylang accounts for the antibacterial activity. Pseduosarsasapogenin-5,20-dien methyl ether was also one of the compounds which was identified in GC-MS analysis. This is a volatile organic compound which may be used for production of methylating agent and also found in some of the plant extracts [15,28,24,22,20]. These compounds have been the cause for potential activity of essential oil of ylang ylang flower in various applications and in some research's, NMR studies had also been done and so the compounds had identified [13].

Larvicidal activity assay: The larvae were left undisturbed for 24 hours at a condition of 26°C with a photoperiod for providing natural conditions. After the 24 hours of incubation, the larvae were taken and checked the mortality. The mortal stage of larvae was confirmed by not having any movement whereas in control, larvae were found alive. Under the microscope, the mortality of larvae was confirmed. This assay confirms the larvicidal activity of essential oil of ylang ylang flower [11].

Conclusion: This study concludes that an essential oil extracted from the flower of ylang ylang *(Artabotrys hexapetalus)* contains various chemical constituents and activities. The UV/Vis spectrophotometer effectively concludes that essential oil contains phosphide bonds which makes the amino acids linked to other to form the proteins. The anti-oxidant property of this essential oil promote this plant as a valuable medicinal value. The anti-microbial activity also does the same and makes it as an efficient drug as antibiotic for many diseases. In the list of eucalyptus oil, rose, lavender, garlic and so on, now this ylang ylang oil of annonaceae family also been added. The term Ylang-Ylang signifies "Blossom of Flowers" and it is really supported. Its therapeutic properties were known to the locals of these islands since long, however were evaded from the cutting-edge world. Afterward, with coming of analysts and researchers of present world into these thick downpour woods and into the lives of the first occupants of these islands, the restorative properties were uncovered and now the market loaded with beautifying agents and other items containing (or possibly utilizing the name) Ylang-Ylang Essential Oil. Because of its higher fragrance it can also be used in aroma therapy and also promotes for cosmetics. Larvicidal activity of essential oil can be used to develop repellent against the mosquito. Still, the obtained result just confirms the presence of larvicidal activity lot more studies have to be made. And so, this study might be continued in the future for converting this essential oil as a valued marketable product in pharma world and aroma therapy.

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