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Influence of time and stage of flower harvest on oil yield, quality and screening of oil for its antibacterial properties of *Cananga odorata* Hook. F. and Thomson

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Abstract
The experiment on influence of time and stage of flower harvest on oil yield, quality and screening of oil for its antibacterial properties of *Cananga odorata* HOOK. F. AND THOMSON. was carried out at the Department of Horticulture, GKVK campus, University of Agricultural sciences, Bangalore. The experiment consists of two seasons (October-December and March-May) with 6 treatment each and with 3 replications and FCRD was followed. In season 1, the highest oil percentage recorded was 1.87% with distillation of flowers at green stage at 12.00 noon during the months October-December. In season 2, the highest oil percentage recorded was 1.27% with distillation of flowers at green stage at 12.00 noon during the months March-May. GC-MS analysis showed that the percentage composition of specific constituents of oil from the present study was comparable with first grade oil of Ylang-ylang as per Madagascar standards. The oil extracted from both the stages of flower showed inhibitory effect against *Bacillus cereus*, *Bacillus subtilis* and *Escherichia coli.*

Keywords: Ylang-ylang, harvesting stage, oil yield, quality and antibacterial properties

Introduction
Ylang-ylang (*Cananga odorata* Hook. F. and Thomson.) is a fast growing, tall evergreen tree belongs to the Annonaceae Family. It is native to South-East Asian islands mainly Malaysia, Indonesia and Philippines. Presently, it is cultivated in many countries such as pacific Islands, Northern Australia, Thailand, Vietnam, Madagascar etc. It has been introduced and cultivated throughout the tropics and subtropics usually as an ornamental tree in gardens. Commercial cultivation of *C. odorata* for the production of oil was started in the Philippines followed by Indonesia. (Yusuf and Sinohin, 1999)

Ylang-ylang is used in different fields like perfume industry, food flavouring industry and Cosmetic industry. It is used in aromatherapy as a sedative, antiseptic, as an aphrodisiac, to treat trauma, acute anxiety, Phobias, relief of depression and stress. Ylang-ylang oil exhibits harmonizing and relaxing effect and inhalation of oil leads to a significant decrease of blood pressure. It is also known for its medicinal properties, to treat stomach ailments, dried flowers used against malaria and fresh flowers are used to treat asthma. Ylang-ylang is also having anti-bacterial, anti-fungal and anti-cytotoxic properties. It contains antioxidant activity which supports its application in cosmetic and in spa as an anti-aging product.

Steam and Hydro distillation are the most common methods of extraction used in Ylang-ylang. Extraction with petroleum ether or benzene is occasionally used. The oils extracted after distillation are quantified using Gas Chromatographic-Mass Spectrometric (GC-MS) analysis.

Composition of Ylang-ylang is aliphatic alcohols, aldehydes, acids, esters, phenols, monoterpenes, sesqiterpenes and their oxygenated derivatives, phenyl propanoids, nitrogen containing compounds as well as benezenoid compounds in the flower essential oils. The main components of Ylang-ylang oils are linalool, geranyl acetate, germacrene-D, β-caryophyllene, benzyl acetate, geraniol, methyl p-cresol, methyl benzoate, farnasene and benzyl benzoate. There is a wide variation in the compositions of ylang-ylang oils and their different grades depending upon the origin and their grade. (Lawrence, 1986) [8].

Materials and Methods
The experiment was carried out at Medicinal and Aromatic crops field, quality analytical laboratory of Department of Horticulture, University of Agricultural Sciences, Gandhi Krishi

Vignana Kendra, Bengaluru, during October to May 2020-2021. The flowers were harvested early morning before 8.00 am at 10 days interval from labelled trees in two seasons (October-December and March-may).Harvested flowers were separated as green and yellow stage flowers and distilled with hydro distillation method using Clevenger's apparatus and oil yield was measured.

Hydro distillation

The method used for extraction of essential oils in this experiment was Hydro distillation. Hydro distillation is the simplest process to extract and isolate the essential oils. This method enables the most delicate fragrance molecules from not being destroyed at the high temperature as in other methods. In the process of distillation, the temperature of the bottom layer of the charge rises first as the steam condenses on the herbaceous mass and in this process, its latent heat is utilized in raising the temperature of the mass and subsequently vaporizing the oil droplets. The temperature of the successive layers of the mass inside the still, gradually starts rising till the temperature in the still equalize.

Gas chromatography- Mass spectroscopy (GC-MS)

GC-MS is an analytical method that combines the features of gas-chromatography and mass spectrometry to identify different substances within a test sample. A GC has a mobile phase and a stationary phase. The mobile phase is nitrogen/helium and the stationary phase is the column. When a sample is injected, then it is carried by the mobile phase across the stationary phase and based on the mobility of different hydrocarbons in the stationary phase, they get separated and are detected at different time intervals.

Determination of Antibacterial activity

The oil extracts from Ylang-ylang (*Cananga odorata* HOOK. F. AND THOMSON.) were tested for their antibacterial activity towards the following human pathogenic gram-positive and gram-negative bacterial cultures, *Bacillus cereus* (MCC 2236), *Bacillus subtilis* (MCC 2183), *Streptococcus agalactiae*(MCC 3039), *Escherichia coli* (MCC 2552), *Pseudomonas aeruginosa* (MCC 3097) and *Shigella flexneri* (MCC 3095) collected from National Centre for Microbial Resource (NCMR), Pune, India. The antibacterial activity was determined by the agar well diffusion assay. Full strength agar plates were prepared and were seeded with the pathogenic bacteria using a sterile spreader. A well of 2mm dia was borne at the centre of the agar plate. The wells were filled with 100µl of the oils extracted. The plates were incubated at 30°C and were observed for the formation of bacterial growth inhibition zone. The diameter of the zones were measured.

Results and Discussion

The observations of Season 1 with respect to Oil percent is presented in Table 1. Significant difference was noticed with respect to time of distillation where, t_3 (distillation of flowers at 12.00 noon) gave the highest oil yield of 1.63% followed by t_1 (distillation of flowers at 8.00 am) which recorded 1.34% and the least oil yield was of 1.24% observed in t_2 (distillation of flowers at 10.00 am). Stage of flowers also showed significant differences, green flower stage gave maximum oil yield of 1.59%compared to yellow flower stage where 1.20% was recorded.

The interaction effects between time of distillation and stage of flower was found to be non-significant. The highest oil percentage recorded was 1.87% when the flowers were distilled at green stage at 12.00 noon followed by 1.53% (Distillation of flowers at green stage at 8.00 am) and 1.38% (Distillation of flowers at green stage at 10.00 am). The least oil percentage noticed was1.09% (Distillation of flowers at yellow stage at 10.00 am).

The observations on Oil percent recorded in Season 2 (March-May) is presented in Table1. Significant difference was noticed with respect to time of distillation where, t_3 (distillation of flowers at 12.00 noon) recorded the highest oil percent (1.18%) followed by t_1 (distillation of flowers at 8.00 am) which recorded 0.79% oil and the least oil yield of0.73% was noticed in t_2 (distillation of flowers at 10.00 am). Stage of flowers also showed significant differences, green flower stage gave maximum oil yield of 1.02% than yellow flower stage (0.78%).

The interaction effects between time of distillation and stage of flower was found to be non-significant. The highest oil percentage recorded was 1.27% when the flowers were distilled at green stage at 12.00 noon followed by 1.10% (Distillation of flowers at yellow stage at 12.00 noon) and 0.93% (Distillation of flowers at green stage at 10.00 am). The least oil percentage was 0.53% noticed when the flowers were distilled at yellow stage at 10.00 am).

The ylang-ylang flowers at different stages of maturity recorded different quantities of essential oil content on distillation. The mature green flowers gave highest oil yield compared to mature yellow flowers. The essential oil content increased when flowers reach maturity due to good growth rate and increased metabolic activity. However, it had resulted in lower yield if flowers were too mature (Muchjajib and Muchjajib 2011) [10]. When flowers were harvested at yellow mature stage, the oil yield found decreased.

Screening of Ylang-ylang oil for its antibacterial properties

The oil extracted from green and yellow flowers of two seasons was studied and are presented in Table 2. The oil samples were tested for antibacterial assay against six human pathogens.

Azithromycin an antibiotic was used as a control. 100µl of extracted oils was filled into the wells and zone of inhibition was recorded in mm. From the assays performed, it was observed that the oil extracted from the green mature flowers of season 1 showed better resistance against *Bacillus cereus* (16.63 mm zone of inhibition), *Bacillus subtilis* (17.60 mm zone of inhibition) and *Escherichia coli* (16.80 mm zone of inhibition) than other bacteria. In season 2 also the oil from green mature flowers showed better resistance against *Bacillus cereus* (17.79 mm zone of inhibition), *Bacillus subtilis* (19.40 mm zone of inhibition) and *Escherichia coli* (17.56 mm zone of inhibition) than other bacterial strains.

The oil extracted from the yellow mature flowers of season 1 exhibited higher resistance against *Bacillus cereus* (19.58 mm zone of inhibition), *Bacillus subtilis* (22.02 mm zone of inhibition) and *Escherichia coli* (16.76 mm zone of inhibition) than other bacteria. Oil of Ylang-ylang of season 2 from yellow mature flowers also exhibited better resistances to *Bacillus cereus* (20.79 mm zone of inhibition), *Bacillus subtilis* (21.06 mm zone of inhibition) and *Escherichia coli* (16.43 mm zone of inhibition) than other bacteria. Oil obtained from yellow stage flower showed higher resistance

to human pathogenic bacteria than green stage flower, this may be due to higher rate of metabolism and development of effective phytochemical compounds.

The essential oils had the ability to control the Firmicute *Bacilli sp*. Both *Bacillus cereus* and *Bacillus subtilis* showed susceptibility to the oils, rest all the pathogens tested negative and proved to be resistant. The antibacterial activity of essential oil, obtained by hydrodistillation of the Ylang-ylang flowers against *S. aureus, E. coli, P. aeurginosa, Citrobacter* spp and *Klepsiella* spp was studied and proved that all the pathogenic species were susceptible to the essential oils at varying degrees. Ylang-ylang plant extract showed 100% inhibition against *K. pneumonia* (100%) (Akter *et al*., 2019) [1]. Kunova *et al*., (2019) [6] worked on antimicrobial activities of *Cananga odorata* flower essential oil, *Citrus reticulate* peel, *Citrus paradise* peel and *Juniperus communis* fruit.

The essential oil obtained from the flower oils tested from the two seasons had the ability to control only *Bacillus spp* and not other bacteria used in thestudy. The control of *Bacillus* spp is quite adequate and are highly complementary to the prior studies conducted. The antibacterial activity of any compound is directly proportional to polarity of the compounds and their intrinsic bioactivity (Anjana *et al.*, 2009) [3]. In Ylang-ylang flowers, compounds such as phenols, geranyl acetate, germacrene-D, geraniol and methyl p-cresol were found and showed antibacterial activity. The antibacterial activity of the essential oils was due to presence of phenols as indicated by Kalemba and Kunicka (2003) [5].

The findings from the study proved to be important because, as per recent reports the *Bacillus spp*have developed resistance to most of the antibiotics developed. *Bacillus cereus* strains showed resistance to most Beta-lectum antibiotics like penicillin and cefotoxin (100%), amoxilin and ampicillin (99.3%*)* (Fielder *et al*., 2019) [4]. Alanber *et al*. (2020) [2] studied the multidrug resistance of *Bacillus* strains causing public health risks in powdered infant milk. Hence, it is important that identification new chemicals for antibacterial therapy against the human pathogens play an important role if the existing drug fails. From the study, it could be observed that the Ylang-ylang flower extracts have the antibacterial property against the human pathogenic *Bacillus cereus, Bacillus subtilis* and *Escherichia coli.* There could be possible inhibition of other pathogens with increased concentration.

Table 1: Interaction effect of time of distillation and stage of flower on oil yield

	Season 1	Season 2
Time	**Mean (%)**	**Mean (%)**
t_1	1.34[b]	0.79[b]
t_2	1.24[c]	0.73[c]
t_3	1.63[a]	1.18[a]
S.Em±	0.06	0.05
CD at 5%	0.19	0.15
Stage		
g	1.59[a]	1.02[a]
y	1.20[b]	0.78[b]
S.Em±	0.05	0.04
CD at 5%	0.15	0.13
Time×Stage		
$T_1=t_1\times g$	1.53	0.87
$T_2=t_2\times g$	1.38	0.93
$T_3=t_3\times g$	1.87	1.27
$T_4=t_1\times y$	1.14	0.71
$T_5=t_2\times y$	1.09	0.53
$T_6=t_3\times y$	1.38	1.10
S.Em±	0.08	0.07
CD at 5%	NS	NS

t_1: Distillation of flowers at 8.00 am
g: Green stage of flower
t_2: Distillation of flowers at 10.00 am
y: Yellow stage of flower
t_3: Distillation of flowers at 12.00 noon

Table 2: Antibacterial properties of *Cananga odorata* oils

Zone of inhibition (mm)						
Stage	**T1**	**T2**	**T3**	**T4**	**T5**	**T6**
GMS-S_1	16.63 (±0.18)	17.60 (±0.18)	-	16.80 (±0.18)	-	-
GMS-S_2	17.79 (±0.18)	19.40 (±0.18)	-	17.56 (±0.20)	-	-
YMS-S_1	19.58 (±0.18)	22.02 (±0.21)	-	16.76 (±0.18)	-	-
YMS-S_2	20.79 (±0.20)	21.06 (±0.18)	-	16.43 (±0.18)	-	-

Gram-positive bacteria: T1- *Bacillus cereus*; T2 - *Bacillus subtilis*; T3 - *Streptococcus agalactae*
Gram-negative bacteria: T4 -*Escherichia coli*; T5- *Pseudomonas aeurginosa*;
T6 – *Shigella flexneri*
GMS-S_1 – Green mature stage oil of season 1(October-December)
GMS-S_2 - Green mature stage oil of season 2 (March-May)
YMS-S_1 - Yellow mature stage oil of season 1(October-December)
YMS-S_2 - Yellow mature stage oil of season 2 (March-May).

Table 3: GC-MS analysis of different chemical constituents present in Ylang-ylang (*Cananga odorata*) as influenced by stage of flower harvest and time of distillation

Seasons	Treatments	Methyl anisole (%)	Linalool (%)	Methyl benzoate (%)	Benzyl acetate (%)	Benzyl Benzoate (%)	Cinnamyl ester (%)
Season 1 (October-December)	T_1	6.84	28.95	4.22	12.26	5.30	4.19
	T_2	6.13	20.23	4.59	11.25	8.92	5.56
	T_3	5.75	23.86	4.54	14.48	5.53	6.12
	T_4	3.58	14.98	3.21	10.67	10.00	5.64
	T_5	3.63	22.10	4.72	15.11	6.53	7.20
	T_6	5.32	21.41	4.54	14.72	8.10	6.25
Season 2 (March-May)	T_1	5.65	17.39	4.53	10.76	7.05	5.16
	T_2	3.88	13.85	3.43	8.60	10.11	5.36
	T_3	4.19	14.48	3.39	7.62	9.62	4.73
	T_4	6.70	18.44	5.25	13.36	5.04	4.73
	T_5	6.75	17.65	5.28	14.25	5.30	4.59
	T_6	6.40	17.03	5.00	13.06	8.92	5.34

The observations on different chemical constituents present in Ylang- ylang (*Cananga odorata*) as influenced by stage of flower harvest and time of distillation is presented in Table 3.Various factors influence the chemical composition and quality of the volatile secondary metabolites being extracted, particularly the extraction method, extraction time and the

flower conditions (Vasundhara *et al*., 2017) [12]. In the present study, percentage composition of specific constituents of oil were comparable with first grade oil of Ylang-ylang as per Madagascar standards. Percentage composition of first grade Madagascar soil are as follows: Methyl anisole (5.75-7.60%), linalool (7.27-18.60%), methyl benzoate (1.73-10.39%), benzyl acetate (3.29-38.55%) and benzyl benzoate (4.34-14.85%).

Conclusion

Present study revealed that, it is preferable to harvest flowers at 8.00 am during green mature stage and preferably distilled at around 12.00 noon for a duration of two and half hours to obtain better oil yield/recovery. The study material analysed using GC-MS indicated that the chemical constituents of the Ylang-ylang oil are comparable with first grade oil as per Madagascar standards. The oil extracted from both the stages of flower showed inhibitory effect against *Bacillus cereus*, *Bacillus subtilis* and *Escherichia coli.*

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