Exploitation of Scented Volatiles and Essential Oil of *Mkilua fragrans* Verdc. for Livelihood Enhancement Among the Coastal Communities of Kenya

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ABSTRACT

Essential oils and volatiles are complex mixtures of biologically active substances used since time immemorial as flavoring agents and constituents of a number of commercial products. The fragrant plant, *Mkilua fragrans* Verdc. forms part of a biodiversity of Keny's coastal forests that has remained under-utilized in a heavily impoverished region. Fragrance from the flowers of the plant have been used for decades alongside *Jasminum L. bignoniaceum* G. Don to enhance the quality of the scent commonly applied as perfume in weddings and other important ceremonies by Swahili, Digo and Arab women. In this study, essential oils of *M. fragrans* were extracted by different procedures and quality of fragrance determined by sensory evaluation. The essential oils were extracted from flowers of *Mkilua fragrans* obtained from Kwale, Kilifi and Mombasa counties by hydro distillation, solvent extraction and effleurage. GC-MS analysis of the extracted oils and volatile compounds from effleurage of fresh *Mkilua fragrans* flowers revealed the presence of esters (16.70%), ethers (14.11%), alcohols (12.87%) and hydrocarbons (11.62%). Esters were in highest proportions in headspace trapping for the three stages with (36.00%), (40.48%) and (47.47%), respectively and also in cold effleurage (16.8%). These results suggest that sesquiterpenes and esters, contribute greatly to the strong and original scent of *Mkilua fragrans*. The stability of the extracts varied from 0 to 52% after 30 days exposure to light under room temperature. These findings provide a basis for a chemical formulation that mimics the *Mkilua* fragrance, which may be exploited in the perfume industry thereby enhancing revenue generation and preservation of the plant.

Keywords: *Mkilua fragrans Verdc., Essential oils, Livelihood enhancement, Coastal communities*

INTRODUCTION

Mkilua fragrans Verdc. *(M. fragrans)* belongs to the Family Annonaceae and is part of a rich forest biodiversity along the coast of Kenya and Tanzania (Muhammed, Pakia & Wainaina, 2014). The plant has been used for decades as a source of perfume by the Swahili and Arab women. According to Verdcourt (1971), *M. fragrans* is identified variably between two Kenya coastal communities as 'Mkilua', 'Mlua' and 'Muua' (Swahili), and 'Mlua', 'Mchilua', 'Mrua' and 'Chingade' (Digo). Apart from the name Chingande from the Digo community, all the other names maintained the prefix M, to denote the recognized growth habit of *M. fragrans* as being a tree ('Mti' and 'Muhi', respectively) and the suffix -ua (flower) (Pakia, Cooke & Van Staden, 2003). *M. fragrans* is sold together with other flowers in a vase as part of decoration during celebrations and ceremonies, including weddings. It is also used in an artistic floral design known as *Kikuba, Kishada, Koja* and *Joho* with a collection of flowers including Ylang ylang, Jasmine, Rose, and Nargis (Muhammed *et al*., 2014). Previous studies indicate that ethanolic extracts of the root and stem bark of *M. fragrans* were found to be very potent against *E.Coli.* Lyantagaye, (2014) scented extracts from fruits and stem barks of *M. fragrans* gave *mkiluynoic* acid 1 and *mkiluynoic* acid 2, which exhibit antifungal activity against *Candida albicans*. (Baraza, Nkunya, Jonker, Juma & Waibel, 2006). Essential oils extracted from the

leaves of *M. fragrans* by hydrodistillation gave limonene, β-elemene and caryophylleneoxide as terpene components (Odalo, 2004). These essential oils were repellent to the mosquito, *Anopheles gambiae* (Odalo *et al*., 2005).

This study as carried out in recognition of the rich biodiversity of the Kenya coastal forest and the increasing threat to these forests, which are partly associated with increased poverty among the local communities. The coastal forests of Kenya are a part of the ''ancient coastal vegetation mosaic'' of eastern Africa, rich in biodiversity, and part of the most important biological systems of the world (Robertson & Luke, 1993). It is also evident from previous studies that there exists untapped biodiversity in this region. There is, therefore, a need to evaluate more plant species as sources of perfume. *M. fragrans* has been in use as a source of perfume for decades. This builds on an already existing local practice, hence would be easily accepted at local level. This in turn is expected to enhance economic empowerment of the local community and especially women who have been at the forefront in the use of *M. fragrans* (Muhammed *et al.*, 2014). The application of *M. fragrans* in perfume has not been fully exploited while alternative potential uses of the scent from the flower such as fragrance for soaps and other toiletries, candles, soft drinks and medicines have not been exploited Muhammed *et al.* (2014) in previous studies, reported that the volatile components of *M. fragrans* were isolated by hydro distillation and solvent extraction. The less pleasant essential oil obtained from the leaves by hydro distillation revealed the presence of camphene, caryophylleneoxide, (-)-dehydroaromadendrene, 4-isopropylbenzenemethanol, limonene and a-ylangene as the major constituents (Odalo, 2004). The methanolic extracts of the flowers revealed the presence of *mkiluaynoic* acid 1 and *mkiluaynoic* acid 2 as the major components (Baraza *et al.*, 2006). The reported data so far has not revealed phytochemical components of the plant's fragrant molecules from effleurage.

MATERIALS AND METHODS

The plant was identified by a botanist and voucher specimen no. HM.NOV./2012/01 is logged at the University of Nairobi Herbarium for future verification. Flowers for extraction by effleurage were collected in two locations namely Mtaani area in Kilifi Town, Kilifi County and Bakarani in Mtopanga, Mombasa County, Kenya. In this study four development stages of the *M. fragrans* flower were identified based on the aroma, floral and petals shape. Extraction by effleurage using virgin coconut oil was done using stage III of the *Mkilua* flower.

Virgin coconut oil was used as a solvent to extract scented volatiles from *M. fragrans* flowers. Both cold and hot effleurage methods were investigated using flower petals of *M .fragrans* Stage III.

Cold effleurage was conducted in two ways either by preserving the oil as well as the flowers according to Putthita *et al*., (2009) with modification where palm stearin was melted at 80^0 C and spread over two frame glass sheets. In this study Virgin coconut oil was used instead of palm stearin and *M. fragrans* flowers soaked in glass bottles. The amount of flowers used ranged from 1000g to 3500g/200mL of palm stearin, while 50g/200 mL of virgin coconut oil were used for *M. fragrans* flowers.

Virgin coconut oil of 200mL was placed in a glass bottle and 50g of *M. fragrans* flowers were added and the mixture left at room temperature (approximately 27^0C) for 48 hours. The flowers were then removed and a clear sample of the virgin coconut oil and Volatile Organic Compounds (VOCs) was withdrawn and set aside for Gas Chromatography Mass Spectroscopy (GC-MS) analysis. Another 50g of fresh flowers were added to the remaining oil, left for 48 hours and liquated again. The procedure was repeated for the third time and another aliquot similarly withdrawn. The three aliquots were analyzed by GC-MS to establish the oil content.

Hot effleurage was done by heating 50 g of flowers/100mL of coconut oil at 60^o C for 30 minutes. The mixture was then cooled and kept in a refrigerator overnight at 4^0C. The oil was then agitated for 3 to 4 days, filtered and finally a clear sample was taken for GC-MS analysis Putthita *et al.* (2009).

Characterization, identification and determination of relative amounts of the components of extracts

from effleurage, was done by gas chromatography-linked with mass spectrometry (GC-MS). The GC-MS machine used was of the order Finnigan GC 8000 series, interfaced with a voyager EI-MS detector (CE Instruments, Milan, Italy) used for separation, detection and quantification of the samples. Column Rtx- 5MS dimensions 20m x 0.25mm x 0.25 µm. Injector temp: 220^{0}C Tempprog-initial temperature of 60^{0}C held for 2 minutes then the temperature ramped at a rate of 18^{0}C/min to 240^{0}C and maintained for 10 minutes. Scanning range: 50-450mz 99.99% pure and the gas flow rate were at 25 Kpa. The compositions of essential oils and volatile compounds were identified using standard procedures adapted from (Odalo *et al.*, 2005). The volatile compounds were identified by comparison of their retention index (RI) relative to (C_6-C_{40}) n-alkanes with those of literature and/or those of authentic standards as adopted from mass spectral libraries and comparison with other published spectral data (Robertson & Luke, 1993).

RESULTS AND DISCUSSION

GC-MS analysis of scented volatile compounds extracted from *M. fragrans* with coconut oil yielded a total of 8 compounds. These included hydrocarbons (54.41%), ethers (16.97%), heterocompounds (12.44%) and esters (7.97%) (Table 1).

Table 1: *Mkilua fragrans* flower from cold effleurage with preserved oil sample

(first to third) Flower sample

SN.	Compound	% Peak Area		
		Sample 1	Sample 2	Sample 3
1.	tetrahydro-2-(1-methylethoxy)-2H-pyran	5.89	7.78	-
2.	1-(ethenylthio)-octane	12.44	15.53	14.11
3.	3-methyltridecane	1.16	0.89	11.60
4.	n-propylheptylether	1.00	-	-
3.	Trans-2,3-bis(1-methylethyl)oxirane	10.08	11.51	0.82
6.	Isopropyl palmitate	9.25	4.13	10.62
7.	2-(2-hydroxyethoxy)-octadecanoate	-	5.93	6.08
8.	1,1-Dodecanediol	0.84	-	-
9.	11-tridecen-1-ol	7.97	11.16	12.87
10	Bis(2-ethylhexyl)phthalate	-	30.94	-
11.	1-(pentyloxy)-2-hexene	-	7.78	-
12.	2,3,7-trimethyldecane	50.26	-	-
13.	3-methyltetradecane	2.95	-	-
14.	O-(2-methylpropyl)-Hydroxylamine	-	-	2.35
15.	3-Hexadecyloxycarbonyl-5-(2-hydroxyethyl)-4-methylimidazolium ion	-	-	1.47
16.	3-methylpentadecane	-	-	0.65
17.	2,6,10,14-tetramethylheptadecane	-	-	13.12
18.	2-methylpentadecane	-	-	1.79

Preserving the extraction oil lead to a general increase in concentration of the components as depicted by enhanced peaks 1,2,3,4 and 5 (Figure 1 and Table1) as compared to the first sample. The GC-MS analysis (Figure 2 and Table 1) of second flower sample gave esters (40.0%), ethers (23.13%), heterocompounds (15.56%), alcohols (12.10%) and hydrocarbons (0.89%). The main compounds were Bis(2-ethylhexyl)phthalate (30.94%), 1-(ethenylthio)-octane (15.56%), trans-2,3-bis(1-methylethyl) oxirane (11.51%), 11-tridecen-1-ol (11.16%), tetrahydro-2-(1-methylethoxy) 2H-Pyran (7.78%) and isopropylpalmitate (4.14%). The presence of additional compounds in the second sample may have been

due to incomplete extraction in sample. Some compounds were missing in this sample and it is thought that they were exhaustively extracted and their concentrations below detectable levels.

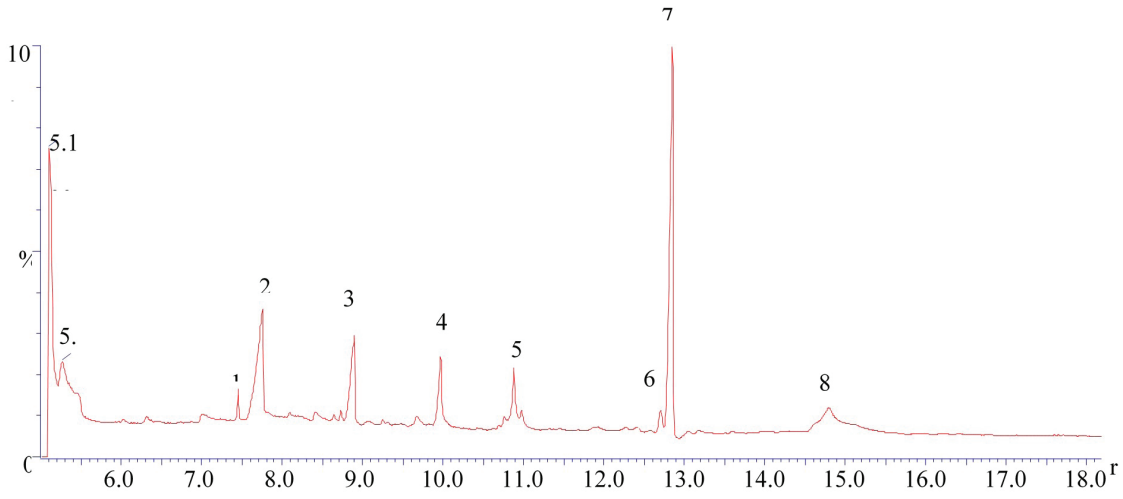

Figure 1: GC-profile of scented compounds from *Mkilua* flowers with preserved coconut oil first flower sample

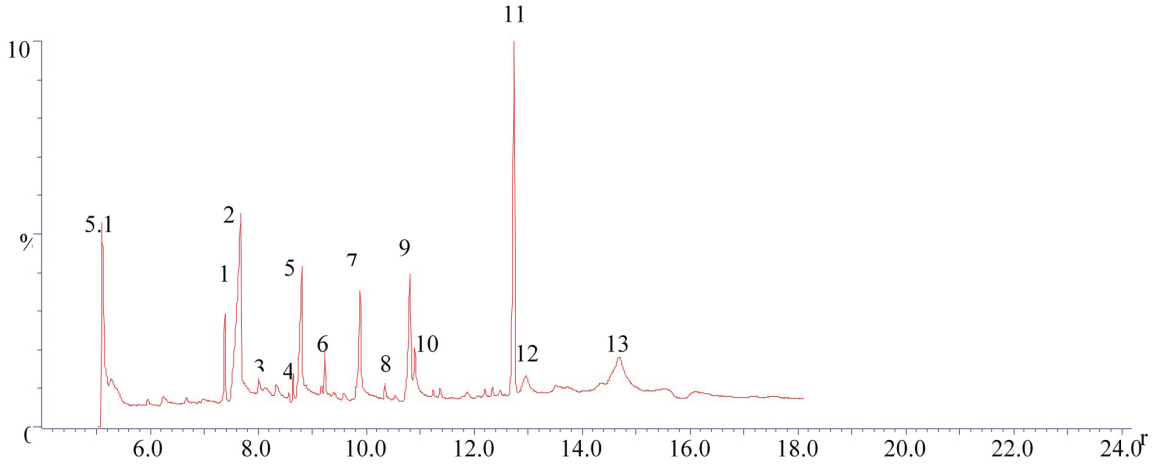

Figure 2: GC-profile of scented compounds from *Mkilua* flowers with preserved coconut oil second flower sample

The extraction of fresh flower samples with the oil used in the second extraction revealed esters (16.70%), ethers (14.11%), alcohols (12.87%) and hydrocarbons (11.62%) by GC-MS analysis. Tetrahydro-2-(1-methylethoxy)- 2H-Pyran was missing in sample 3 while new compounds were extracted such as 3-hexadecyloxycarbonyl-5-(2-hydroxyethyl)-4-methylimidazolium ion and O-(2-methylpropyl)-Hydroxylamine. The main compounds were 1-(ethenylthio)-octane (14.11%) 2, 6, 10, 14-tetramethylheptadecane (13.12%), 3-methyltridecane (11.60%) and Isopropyl palmitate (10.62%). (Figure 3 and Table 1).

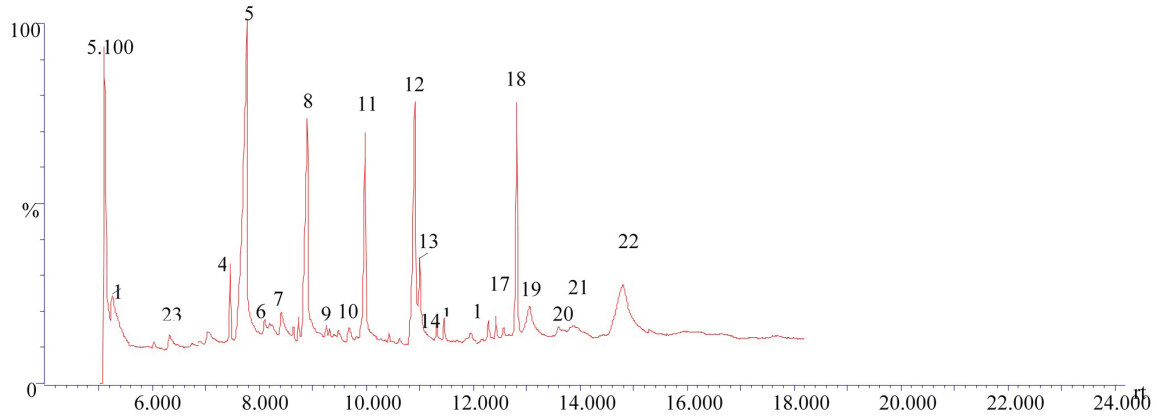

Figure 3: GC-profile of scented compounds from *Mkilua* flowers with preserved coconut oil third flower sample

There was a cumulative extraction of scented volatile compounds by coconut oil making it a suitable solvent for extracting the stated compounds thus qualifying its use for decades by the local Swahili and Arab women in extracting scented volatile molecules from *M. fragrans* flowers. It is evident that sample 3 was more saturated and contained a high proportion of the scented volatile compounds than in sample 2 and 1. This extraction by preserving oil helps to obtain a more scented and concentrated oil extract. Several additional compounds were also obtained from sample 1 to 3. The oil was also more scented than the one extracted from same flower and oil sample. The presence of additional compounds in sample 2 and 3 suggests that the oil/flower ratio was limiting and may not have been optimal for complete extraction of all the scented VOCs.

CONCLUSION AND RECOMMENDATONS

This study established that scent from the *Mkilua fragrans* flower can now be associated with both qualitative and quantitative aspects of the VOCs extracted by effleurage. The fragrance is attributed to the high proportions of esters in the Mkilua oil. The plant should be preserved and artificial fragrance be developed based on established chemical components.

ACKNOWLEDGEMENT

Authors are grateful to the National Council for Science, Technology and Innovation (NACOSTI) and the Technical University of Mombasa (TUM) for the financial support, Pwani University (PU) and Technical University of Mombasa (TUM) for providing the laboratory space and Jomo Kenyatta University of Agriculture and Technology (JKUAT) for availing equipment for chemical analysis.

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