EVALUATION OF THE YIELD AND THE ANTIMICROBIAL ACTIVITY OFTHE ESSENTIAL OILS FROM: *Eucalyptus globulus*, *Cymbopogon citratus* and *Rosmarinus officinalis* IN MBARARA DISTRICT (Uganda)

EVALUACION DEL RENDIMIENTO Y LA ACTIVIDAD ANTIMICROBIANA DE LOS ACEITES ESENCIALES DE *Eucalyptus globulus*, *Cymbopogon citratus* and *Rosmarinus officinalis* EN EL DISTRITO DE MBARARA (Uganda)

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Abstract

Essential oils constitute a relatively common group of natural products present in aromatic medicinal plants. They are volatile liquids usually with pleasant and sometimes intensive odors (aroma).In Mbarara District (Uganda) there are different plants that produce essential oils but no particular study has been done to state the yield and the evaluation of the antimicrobial activity of those essential oilsThe country is been deforested very fast because the people need the wood as oil so to demonstrate that those Essential oils could afford biological activities useful for the treatment of infections may alert the preservation of those species of plants.The yields of the essential oils obtained in the present study were: R. oficcinalis 1,005 % similar to literature. C. citratus 0,645 % higher than literature. E. globules 0,205 % lower than literature. The three essential oils showed antimicrobial activity against the three micro organisms tested over 40 % in comparison with the standard drugs used as positive test and it is important to note that dilutions of the essential oils were more active than the pure ones.

Key Words: Essential Oils, *Cymbopogon citrates, Rosmarinus officinalis, Eucalyptus globules,* antimicrobial activity.

Resumen

Los Aceites Esenciales constituyen un grupo de metabolitos secundarios comúnmente presentes en plantas aromáticas. Son líquidos volátiles de gran complejidad química generalmente con olores agradables a veces intensos

llamados aromas. En el Distrito de Mbarara (Uganda) crecen diferentes plantas que producen Aceites Esenciales pero, no se informan estudios científicos que avalen el rendimiento de los mismos y/o su efecto antimicrobiano. El País está sufriendo una deforestación acelerada pues las personas utilizan la madera como combustible barato y accesible por lo que demostrar que los Aceites Esenciales pueden ser una fuente de tratamiento para enfermedades infecciosas puede favorecer la preservación de algunas especies en el territorio. Con el presente trabajo se demuestra que los rendimientos de los Aceites Esenciales de tres plantas son los siguientes: *R. oficcinalis* 1,005 % similar a los informes de la literatura, *C. citratus* 0,645 % superior a los informes de la literatura y *E. globulus* 0,205 % inferior a los informes de la literatura. Los tres Aceites Esenciales mostraron *in vitro* actividad antimicrobiana del 40 % frente a los tres microorganismos evaluados observándose que las diluciones de los mismos fueron más activas que los aceites puros evaluados.

Palabras clave: Aceites Esenciales, *Cymbopogon citrates, Rosmarinus officinalis, Eucalyptus globules,* actividad antimicrobiana.

Introduction

Essential oils constitute a relatively common group of natural products present in aromatic medicinal plants. They are volatile liquids usually with pleasant and sometimes intensive odors (aroma). They also are referred to as volatile oils, ethereal oils, or essences of many plants. The chemical composition is quite different from one plant to another and the main chemical component determines the aroma and its biological activities. Therapeutically, they exert a wide spectrum of biological activities such as: antiseptic (to avoid infections), stimulant, carminative, diuretic, anthelmintic, analgesic and many others according to the chemical composition (IKAM ,1969).

In Mbarara District (Uganda) there are different plants that produce essential oils but no particular study has been done to state the yield and the evaluation of the antimicrobial activity of those essential oils. In the District, infections of the skin and wounds are common and thus the reason for this study to be undertaken is to evaluate if these essentials oils bear antimicrobial activity to be used as an alternative treatment for external infections.

About 80 percent of the population of Uganda relies on traditional medicine because western-trained medical personnel are limited or not really accepted by the community, and traditional healers are easily consulted, living in the same community. The country imports most of its drugs from abroad and often experiences serious shortages. That points to the demand for Traditional Medicine Practitioners (TMPs) for medicinal plants and the fact that the majority

of the people, rural and urban alike, depend largely on herbal medicines for treating a variety of diseases. This reliance is mainly due to the high cost of conventional medicine and inaccessibility of modern health care facilities in most areas. WHO (2002-2005).

An essential oil is a concentrated, hydrophobic liquid containing volatile aroma compounds from plants. They are also known as volatile or ethereal oils. The volatile or essential oils correspond to a mixture of hemiterpenoids, monoterpenoids and some sesquiterpenoids that are in conjunction to oil. The mixture is highly volatile when exposed to air at room temperature thus the name ethereal oils. The odor and taste of an essential oil is determined by the main chemical constituent of the mixture. They are almost insoluble in water and soluble in alcohol and usually lighter than water. They have high refractive index and many of them are optically active. Essential oils are generally extracted by distillation.

Eucalyptus globulus is an evergreen tree. The leaves are steam distilled to extract Eucalyptus oil. The oil has therapeutic, perfumery, flavoring, antimicrobial and biopesticide properties. Eucalyptus is used internally and externally as an expectorant, and to treat infections and fevers. It is also used topically to treat sore muscles and rheumatism. A topical combination of Eucalyptus and Peppermint shows promise as an analgesic. The primary component of Eucalyptus oil is the volatile substance 1, 8-cineol (cineole). (http://www.thefreelibrary.com/Eucalyptus)

Rosmarinus officinalis (Rosemary) is a woody, perennial herb with fragrant evergreen needle-like leaves. It is native to the Mediterranean region. Currently in Uganda, Rosemary is mainly used as a memory booster in the form of a powder and as a flavoring agent in food stuffs. Large-scale production is just starting to boom but it was previously grown on a small scale. The parts of the plants used in medicine are the flowering tops, which have a powerful diffusive, camphoraceaous odor, and an aromatic, bitter taste. They yield their properties to water or spirits but more effectively to alcohol. The leaves behave in a similar manner. Age and dying impair odor and properties, which are due to volatile oils. The plant leaves are also chewed by patients who have cough.

Cymbopogon citratus, commonly known as lemon grass as well as oil grass, is a tropical plant. It is a tall perennial grass. Its leaves are used to make tea which can relieve stomach and gut problems. It can also act as an antidepressant and as a mood enhancer. *Cymbopogon citratus* contains active ingredients like myrcene, an antibacterial and pain reliever, citronellal, citronellol and geraniol.

It is estimated that about 80% of the world population residing in the vast rural areas of the developing and under developed countries still rely mainly on

medicinal plants. Medicinal plants are the only affordable and accessible source of primary health care for them, especially in the absence of access to modern medicine facilities. Studies reveal that there are more traditional medicine providers than the allopathic providers especially in the rural areas WHO (2002-2005)

Ugandans have greater access to traditional than to western health care. Traditional Healers are an integral part of the local culture and are appreciated as key and sustainable sources of care and knowledge on disease and illness. (The Cross-Cultural Foundation of Uganda, 2008). In Mbarara District (Uganda) there are different plants that produce essential oils but no particular study has been done to state the yield and the evaluation of the antimicrobial activity of those essential oils.

The spread of drug-resistant pathogens is one of the most serious threats to successful treatment of microbial diseases. Throughout the ages essential oils and other extracts of plants have evoked interest as sources of natural products. They have been screened for their potential uses as alternative remedies for the treatment of many infectious diseases. The World Health Organization (WHO) noted that the majority of the world's population depends on traditional medicine for primary healthcare. Medicinal and aromatic plants are widely used as medicine and constitute a major source of natural organic compounds. **(**SEENIVASAN *et al*., 2006).

A survey of the use and value of medicinal plants and other traditional medicine in Kasese District, Uganda is slowly being conducted. 492 members of support groups and health-care clinics was undertaken in a group setting: 23 groups with a mean size of 21, age range 4-53, which represented 0.1% of the population, covering nine of the 20 sub-counties, both in the native languages and in English, using photographs and specimens of 12 plants. Most admitted to using plants at some time; 81% for self, 77% for their children; 45% admitted to using traditional healers as a source for information about health. Most plants were home grown or available locally. Medicinal plants were used for respiratory infections, fever, malaria and diarrhea/vomiting (CHALLAND, 2005).

Materials and methods

Plant materials

Fresh plant leaves of *Eucalyptus globulus, Cymbopogon citratus* and *Rosmarinus officinalis* were each collected in the same month (March 2009), one hour before distillation of the essential oil, from the garden of Mbarara

University of Science and Technology (**MUST)**. The plant leaves were taken to the Pharmacy Laboratory of Mbarara University of Science and Technology for the extraction. All plant material was authenticated in the Biology Department of Mbarara University of Science and Technology (Fig.1).

Figure 1. Plants of *Eucalyptus globulus* (A), *Cymbopogon citratus* (B), *Rosmarinus officinalis* (C).

Procedure for preparation of essential oils

The distillation apparatus was a Clevenger one.

Each of the three plants was collected early in the morning at about 07:00 before the sun was high in the sky on the day scheduled for extraction of the essential oil. *Cymbopogon citratus* was collected first, followed by *Rosmarinus officinalis* on the following day and finally *Eucalyptus globulus* on the last day and in the same week of March 2009. The leaves were cut out by a pair of scissors with a small part of the supporting branch from the Garden of Mbarara University of Science and Technology. The plants were then taken to the laboratory in a plastic bag. The leaves were cut to smaller pieces by scissors to increase the efficiency of the extraction and thereby increasing the yield to be obtained. The cut leaves were then measured using an electronic measuring balance and were put into the round bottom flask of the equipment with sufficient water and set up into the rest of the apparatus.

For *Rosmarinus officinalis* however the leaves were already small there was no need for further reduction of the size by cutting, only detaching them from the supporting branch by a firm sliding motion of the hand. The heating mantel was switched on and the extraction process was set to start. After about one hour the 1st extraction was obtained in the collecting column, the quantity of the oil is read off directly from the graduated column and recorded. The used leaves are

then poured out and a fresh set of leaves is used and once again the process was repeated for another batch of leaves and so on.

For the proceeding batches of the same plant the water used in the 1st extraction process was not poured out, instead was retained and used for the next batch of the same plant because it was saturated in the 1st procedure, this further increases the efficiency of the process and consumes less time. The essential oil is collected by controlling of a tap attached to the collecting column, the water is drained 1st then the top later containing the essential oil was collected in small bottles, covered, labeled and stored in the fridge, to avoid evaporation of the essential oil, ready for antimicrobial testing.

The condensed vapors appeared in two layers where the lower layer was water and the upper was the essential oil layer. The essential oils were measured directly from the apparatus to calculate the yield and then decanted off into the storage bottles.

The essential oils were then taken for antimicrobial tests in the Microbiology Laboratory of Mbarara University of Science and Technology.

Antimicrobial tests

Test microorganisms were obtained from the Department of Microbiology of Mbarara University of Science and Technology and were:
1- *Staphylococcus aureus* (ATCC 25923),

2- Candida albicans (was an isolate from a patient) and

3- *Escherichia coli* (ATCC 25922)

Control antibiotics (Gentamicin for the bacteria and Nystatin for the fungi) were used.

Preparation of stock solutions

The stock solutions of ethanol and the essential oil were made by dissolving 1ml of the essential oils in 1ml of ethanol. From the stock solutions serial dilutions were made to obtain the test solutions of concentration ½, 1/4, 1/8, 1/16, 1/32, 1/64, 1/128. The neat (pure Essential Oil) was also included in the tests.

Procedure for the antimicrobial tests

The method used was well diffusion method.

Candida albicans were incubated on Saborid Dextrose Agar (SDA), *Staphylococcus aureus* and *Escherichia coli* were grown on Mueller- Hinton agar which had been spread with microorganisms at a density adjusted to 0.5 McFarland standards (108 colony-forming units [CFU]/ml).

Antimicrobial assay: The antimicrobial assay was performed by agar diffusion method. The agar was melted, poured on to the Petri dish and left to solidify. Using sterile swabs, the organisms were smeared on the solidified agar.

For agar well diffusion method, a well was prepared in the plates with the help of cork-borer (6mm). 100µl of different concentrations of the test compound was introduced into the wells. The plates were incubated for 24 hours at 37ºC.

Candida albicans was incubated for seventy two hours at 27ºC.

For each microbial species, negative control was maintained where 100µl of ethanol alone without the drug was used for ethanol extract. Also, conventional drugs were used for positive controls. In the central hole of the different Petri dishes, the control was put for each organism, Gentamicin for *E. coli* and *Staphylococcus aureus* and Nystatin for *Candida albicans.*

The results were recorded by measuring the diameter of the zones of growth inhibition surrounding the wells (cylinders). The net effect of the drug extracts was obtained by subtracting the diameter of the zone of inhibition due ethanol alone from the diameter of the zones of inhibition due to the drug extract plus ethanol (BIAVATI *et al*., 2008).

Results

The yield of essential oil from the plant material (Tables 1a, 1b, 1c):

Table 1a. The yield of the essential oils from *Cymbopogon citratus*

	First Extraction	Second Extraction
Weight of Leaves (g)	184	160
Volume after one hour of distillation (ml)	1.1	1.1
Percentage yield (% ml/g)	0.60	0.69
Average yield (% ml/g)	0.645	

Table 1b. The yield of the essential oils from *Rosmarinus officinalis*

	First Extraction	Second Extraction
Weight of Leaves (g)	84	107
Volume after one hour of distillation (ml)	0.75	1.2
Percentage yield (% ml/g)	0.89	1.12
Average yield (% ml/g)	1.005	

Table 1c. The yield of the essential oils from *Eucalyptus globulus*

	First Extraction	Second Extraction
Weight of Leaves (g)	296	240
Volume after one hour of distillation (ml)	0.6	0.5
Percentage yield (% ml/g)	0.20	0.21
Average yield (% ml/g)	0.205	

Antimicrobial activity results

Table 2a. Results for the antimicrobial activity of *Cymbopogon citratus* essential oil

	Zone of inhibition/mm					
Serial Dilution	*Staphylococcus aureus*		*E.coli*		*Candida albicans*	
	Alcohol dilutions	Dilutions	Alcohol dilutions	Dilutions	Alcohol dilutions	Dilutions
½	0	No growth	0	26	0	No growth
¼	0	No growth	0	24	0	No growth
1/8	0	No growth	0	24	0	No growth
1/16	0	16	0	22	0	No growth
1/32	0	28	0	15	0	No growth
1/64	0	18	0	14	0	No growth
1/128	0	12	0	10	0	No growth
Gentamicin	-	58	-	40	-	No growth
Nystatin	-	-	-	-	17	No growth
Neat	-	50	-	18	-	No growth

Table 2b. Results for the antimicrobial activity of *Rosmarinus officinalis* essential oil

	Zone of inhibition/mm					
Serial Dilution	*Staphylococcus aureus*		*E. coli*		*Candida albicans*	
	Alcohol dilutions	Dilutions	Alcohol dilutions	Dilutions	Alcohol dilutions	Dilutions
½	0	36	0	24	0	26
¼	0	48	0	30	0	20
1/8	0	40	0	24	0	18
1/16	0	12	0	12	0	14
1/32	0	8	0	10	0	9
1/64	0	0	0	6	0	9
1/128	0	0	0	6	0	8
Gentamicin	-	?	-	40	-	-
Nystatin	-	-	-	-	17	24
Neat	-	46	-	24	-	20

Table 2c. Results for the antimicrobial activity of *Eucalyptus globulus* essential oil

	Zone of inhibition/mm					
Serial Dilution	*Staphylococcus aureus*		*E. coli*		*Candida albicans*	
	Alcohol dilutions	Dilutions	Alcohol dilutions	Dilutions	Alcohol dilutions	Dilutions
½	0	32	0	32	0	60
¼	0	38	0	29	0	16
1/8	0	22	0	18	0	26
1/16	0	10	0	18	0	14
1/32	0	9	0	14	0	10
1/64	0	9	0	12	0	10
1/128	0	8	0	12	0	9
Gentamicin	-	58	-	40	-	-
Nystatin	-	-	-	-	-	30
Neat	-	32	-	16	-	44

Discussion

The yield of each essential oil was calculated and represented in Tables 1a, 1b and 1c. It is clear from the results that different plants containing essential oils have different quantities or yield of essential oils and even within the same species of plants the exact yield is not reproducible, but is more or less similar. It is therefore not correct to compare the yields between the three plant species but for purposes of presentation *Rosmarinus officinalis* had the highest yield with an average of 1.005 % ml/g yield under the same conditions (duration of extraction, working temperature adjusted by a knob on the heating mantel, environment followed by *Cymbopogon citratus* with an average yield of 0.645 % ml/g and then finally *Eucalyptus globulus* with an average yield of 0.205 % ml/g.

Antimicrobial activity of the essential oil from *Cymbopogon citratus*: *Staphylococcus aureus*: the neat had an effect of 87 % in respect to the standard Gentamicin and the dilution 1/32 had the best effect with 48 % in respect to the standard. *E. Coli*: the neat had an effect of 45 % in respect to the standard Gentamicin and the dilutions were all more active up to 1/16 with 55 % of the activity. *Candida albicans*: the assays were repeated three times and the microorganism did not grow. It is possible to note that the same microorganism was used for the other assays and growed very well, so it is possible that the concentration of the vapor inside the Petri dish is very active and avoid the growth of the strain.

Antimicrobial activity of the essential oil from *Rosmarinus officinalis*:

Staphylococcus aureus: the neat and the three upper dilutions were very active but the standard inhibition of Gentamicin was not obtained therefore was not available for comparison. *E. Coli*: the neat had an effect of 60 % in respect to the standard Gentamicin and the dilutions ½, ¼, 1/8 almost had the same

effect but ¼ had an inhibition of 15 % above the neat. *Candida albicans*: the neat had an effect of 83 % in respect to the standard Nystatin and the first dilution ½ had an inhibition of 30 % above the neat.

Antimicrobial activity of the essential oil from *Eucalyptus globules*: ***Staphylococcus aureus***: the neat had an effect of 55 % in respect to the standard Gentamicin and the dilutions ½, ¼, 1/8, had similar and good activity but dilution ¼ had an inhibition of 18 % over the neat. *E. Coli*: the neat had an effect of only 40 % in respect to the standard but the dilutions ½, ¼, 1/8 and 1/16 gave a higher activity and ½ doubled the activity in respect to the neat and 80 % inhibition in respect to the standard Gentamicin. *Candida albicans*: The neat was more active than the standard Nystatin by an inhibition of 46 % but the dilution ½ doubled the activity in respect to the standard.

In conclusions of this study it is possible to state that the three essential oils bear antimicrobial activity variable according to the microorganisms and in all of them the alcohol dilutions were more active than the neat, maybe because there was better diffusion in agar or because of less loss due to evaporation during incubation time and its higher temperature.

References

BIAVATI, R.B.; PICEAGLIA, M.; MAROTTI, A. 2008. Antimicrobial activity of plant essential oils'. Dep. Of Agro environmental Science and Technology University of Bologna. From Google.co.ug. March 2008.

CHALLAND, S. 2005. A survey of the use of medicinal plants and other traditional medicine in Kasese District, Uganda. Tropical Doctor 35 (4): 222-224.

IKAM, R.1969. *Natural Products: a laboratory guide*. Chapter V. Elsevier Publication, London.

SEENIVASAN, P.; MANICKKAM, J.; SAVARIMUTHU I. 2006. *In vitro* antibacterial activity of some plant essential oils. *BMC Complementary and Alternative Medicine* **6:**39. Extracted from www.biomedcentral.com

THE CROSS-CULTURAL FOUNDATION OF UGANDA. 2008 Promoting Herbal Medicine in Uganda) Extracted from www.crossculturalfoundation.or.ug.

WHO (2002-2005) Traditional Medicine Strategy.