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COMPARATIVE CHEMICAL AND ANALGESIC PROPERTIES OF ESSENTIAL OILS OF *CYMOPOGON NARDUS* (L) RENDLE OF BENIN AND CONGO

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Abstract:

The chemical and analgesic comparison of essential oils of *Cymbopogon nardus* (L) Rendle of Benin and Congo was investigated. The Chemical analysis was carried out by using GS/MS for identification of components of the two essential oils while acetic acid-induced writhings, hot plate and tail flick test models were used for analgesic activity. The results showed that the two essential oils exhibited comparable activity on acetic acid-induced writhings, however, the essential oil of Benin induced more significant effect on hot plate model while the Congolese species showed more effect in the tail flick test. These observations could be explained by some qualitative and /or quantitative differences observed between the constituents of the two essential oils studied.

Keywords: *Cymbopogon nardus*, Essential oil, Chemistry, Analgesic, Comparison, Benin, Congo.

Introduction

Cymbopogon nardus (*C. nardus*) (L) Rendle, a Poaceae is a medicinal plant widely used as culinary (Konwar and Gohain, 1999) and for perfumery (Furukawa, 1918). The Chinese use the leaves more specifically for rheumatism and other uses in the treatment of fever, intestinal parasites, digestive and menstrual problems. *C. nardus* produced a yellow essential oil which presents some pharmacological properties as antifungal (Awuah, 1999), repellent against mosquito (Ansari and Razdan, 1995; Ranaweera and Dayananda, 1996). Literature is poor on the analgesic effect and African comparative study of chemical compounds of essential oil of this plant. The aim of the present study is to compare the chemical and analgesic properties of essential oils of *C. nardus* of Benin with those of Congo.

Material and Methods

Plant material

Leaves of *C. nardus* collected from Loufoulakari, Brazzaville in the department of Pool (Congo) and from Cotonou (Benin) vouchers number Kami 1501 were botanically identified in the Centre d'Etudes sur les Ressources Végétales (CERVE) of Brazzaville (Congo).

Chemical tests

C. nardus leaves of Benin and Congo were subjected to hydrodistillation to obtain essential oils. The components of these two essential oils were identified by GC/MS by direct comparison with reported spectra or by comparison with authentic compounds (Von Sidow et al., 1970; Jennings and Shibamoto, 1980; Nasada, 1980; Adams, 1989; Mc Lafferty and Staufe, 1989; Kondojoyan and Berdague, 1996).

Animals

Male and female BALB/C mice, 2-3 months old, were used for all experiments. Animals were maintained at 22-24 °C with a 12 h light-dark cycle. They were allowed to standard laboratory feed and water ad libitum. For experiments, five mice were used by group. the experimental procedures were carried out in accordance of the ethical guidelines for investigations of experimental pain in conscious animals (Zimmermann, 1983”

Pharmacological tests

Mice were orally administered with the essential oil (1-12 ml/kg) and were macroscopically observed for 6 h. Mortality in each group was observed for 24 h (Lorke, 1983).

Effect on acetic acid induced writhings

Analgesic activities of essential oils of *C. nardus* obtained from Benin and Congo were studied according to Koster *et al.* (1959). Six groups of mice were used. The first group (control) received orally distilled water 5 ml/kg. The third and fourth groups were administered emulsion of essential oil from Benin at doses of 2 and 4 ml/kg respectively while the fourth and fifth groups were administered essential oil of *C. nardus* obtained from Congo at doses of 2 and 4 ml/kg respectively. All the doses were administered orally. The sixth group served as positive control group and were administered paracetamol (50 mg/kg, orally). 1 h after drugs administration, acetic acid 0.6%, 10ml/kg was injected intraperitoneally and the writhings exhibited by each animal were counted for 10min, beginning 10min after acetic acid injection.

Effect on hot plate test

The method of Hikino et al. (1985) was used with slight modification. Mice was placed on a hot plate (56±1°C) and the reaction time (delay to lick the paw) to thermal stimulus was observed with a cut off time of 60 s. Essential oils (2 and 4 ml/kg, orally), morphine (Muscotin®, 2 mg/kg, orally) and distilled water (0.5 ml/kg, orally) were administered 1 h before the test.

Effect on tail flick test

Tail of mice was placed in hot water (56 ± 1°C) and the reaction time (delay of the withdrawal of tail) (Parimala et al., 2003) to thermal stimulus was observed with a cut off time of 10 s 1 hour after administration of products as stated in the hot plate test.

Statistical analysis

Results were expressed as mean ± S. E. M. Student's t - test was used to analyse significance of the results.

Results

Chemical study

The results of chemical analysis of the essential oils of *C. Nardus* of Benin and Congo are presented in

Table 1. The more important constituents are monoterpenes (more than 91% in essential oil of Congo and 86% in those of Benin) represented by citronellal and geraniol (respectively 41.3 and 23% in essential oil of Benin versus 37.5 and 29.4 % in those of Congo. These monoterpenes are associated with their acetates (4.4 and 1.7 in essence of

Benin versus 1.5 and 1.8% for sample of Congo). Oxygen containing compounds were also abundant and are adjoining 90% of total essences. The proportions of aldehydes are high (40% in average). Some sesquiterpenes are present in these preparations, represented by α - farnesene (1.5%) and elemol (4.8%) in specie of Benin and by β -elemene (1.5%), trans- β -caryophyllene (2.5%) and δ -cadinene for congolese essential oil. Hydrocarbons (myrcene and limonene exclusively) represents only 3%.

Table 1: Chemical constituents of essential oils of *Cymbopogon nardus* of Benin and Congo

Order number	Retention indice	Constituents	Percentage	
			Benin	Congo
01	991	myrcene	-	0.1
02	1029	limonene	3.1	2.9
03	1110	linalol*	0.6	0.9
04	1150	isopulegol*	-	0.1
05	1155	citronnellal**	41.3	37.5
06	1226	nerol*	-	0.2
07	1235	citronnellol*	9.2	7.5
08	1247	neral**	-	0.7
09	1260	geraniol*	23.4	29.4
10	1283	geranial**	2.1	1.2
11	1360	citronnellyle* acetate	4.4	1.5
12	1360	eugenol	-	8.1
13	1387	geranyle* acetate	1.7	1.8
14	1400	-elemene	-	1.5
15	1427	trans- -caryophyllene	-	2.5
16	1487	germacrene D	0.5	-
17	1500	bicyclogermacrene	0.4	-
18	1506	-farnesene	1.5	-
19	1520	-cadinene	-	1.1
20	1543	elemol*	4.8	-
21	1576	D-germacrene-4-ol*	1.3	-
22	1616	-eudesmol*	0.3	-
23	1636	-cadinol*	0.4	-
24	1640	-cadinol*	0.3	-
25	1725	trans farnesol*	0.9	-
Total identified compounds :			96.2	97.0
Monoterpenic compounds :			85.8	91.9
Sesquiterpenic compounds :			10.4	5.1
* Oxygen contained compounds :			90.7	88.9
** Identified aldehydes			43.4	39.4

Constituents are classified according to their elution order on DB-5

Table 2: Effect on essential oils of *C. nardus* on acetic acid-induced writhing in mice

Treatment	Dose (p.o.)	Number of writhings	Inhibition (%)
Control (distilled water)	0.5 ml/kg	57.60 ± 1.60	-
EOCNB	2 ml/kg	34.00 ± 2.02*	40.97
	4 ml/kg	30.20 ± 1.32*	47.56
EOCNC	2 ml/kg	36.20 ± 0.73*	37.15
	4 ml/kg	28.00 ± 1.48*	51.38
Paracetamol	50 mg/kg	20.60 ± 2.03*	64.23

^aValues are mean ± S.E.M; n =5; *P <0.001 vs control, student's t-test;
EOCNB: Essential oil of *C. nardus* ; EOCNC: Essential oil of *C. Congo*

Table3: Effect on essential oils of *C. nardus* on time reaction in hot plate test

Treatment	Dose (p.o.)	Reaction time (sec)
Control (distilled water)	0.5 ml/kg	3.80 ± 0.37
EOCNB	2 ml/kg	35.80 ± 5.97*
	4 ml/kg	46.60 ± 5.02*
EOCNC	2 ml/kg	31.60 ± 1.43*
	4 ml/kg	38.00 ± 4.39*
Morphine	2 mg/kg	59.60 ± 4.00*

^a Values are mean ± S.E.M; n=5; *p< 0.05 vs control, student's t-test ;
EOCNB: Essential oil of *C. nardus* ; EOCNC: Essential oil of *C. Congo*

Table 4: Effect of essential oils of *C. nardus* on reaction time in tail flick test

Treatment	Dose(p.o)	Reaction time (sec)
Control (distilled water)	0.5 ml/kg	1.80 ± 0.37
EOCNB	2 ml/kg	2.80 ± 0.37 [#]
	4 ml/kg	2.80 ± 0.73 [#]
EOCNC	2 ml/kg	3.53 ± 0.73 [#]
	4 ml/kg	6.20 ± 0.91**
Morphine	2 mg/kg	6.20 ± 0.66 [#]

^a Values are mean ± S.E.M; n=5; [#]p< 0.05 , **p< 0.001
EOCNB: Essential oil of *C. nardus* ; EOCNC: Essential oil of *C. Congo*

Acute toxicity

There was no toxic effect observed with the two essential oils until the dose level of 12 ml/kg.

Effect of essential oils of *C. nardus* of Benin and Congo on acetic acid- induced writhings

As shown in Table 2, essential oils of *C. nardus* of Benin and Congo exhibited comparable analgesic activities on acetic acid-induced writhings in mice. These effects were dose-dependent for each essential oil. However, it was less than that of the positive control product and paracetamol.

Effect of essential oils of *C. nardus* of Benin and Congo on hot plate test

The two essential oils increased significantly the reaction time in the hot plate test (Table 3). The most significant effect was observed with the dose of 4 ml/kg of essential oil of Benin even though it was less than that of morphine at the dose of 2 mg/kg.

Effect of essential oils of *C. nardus* of Benin and Congo on tail flick test

Table 4 shows the effects of essential oils on reaction time in the tail flick test. There was a significant and dose-dependent activity of essential oil of *C. nardus* of Congo. The dose of 4 ml/kg showed the same effect as that of the reference drug (morphine). However, the slight analgesic effect of essential oil of Benin, which was observed only at the high dose used (4 ml/kg), was less than that of Congolese specimen at the dose of 2 ml/kg.

Discussion

The two essential oils studied present some qualitative and quantitative chemical differences by the nature of the identified constituents. For example, eugenol is present in Congolese specie (8.1%) but this compound is absent in the sample of Benin; elemol is identified in Beninese specie (4.1 %) but it is absent in those of Congo. The nature of the most important compounds identified shows that these essences are similar to those of Bangladesh (Nigam and Datta, 1973; Manzoor-I-Khuda *et al.*, 1984) but, different from those of Zimbabwe (Moody *et al.*, 1995) which is rich in geraniol (29.5%) and geraniol formate (8.8%). Essentials oils of *C.nardus* of Benin and Congo are well tolerated and these results confirm the safety of this preparation as earlier reported (Der Marderosian *et al.*, 2004). Literature survey on analgesic effect of *C. nardus* is poor. The three analgesic tests used show that the two essential oils possess analgesic activity. In acetic acid-induced writhings and hot plate tests, the two essential oils exhibited comparable dose-dependant activity. However, as shown in the hot plate test, a more significant effect was observed with essential oil of Benin while the Congolese preparation was more effective in the tail flick method. These observations could be explained on the basis of some qualitative and/or quantitative differences observed between the constituents of the two preparations.

Conclusion

These results confirm the analgesic properties of *C.nardus* as used ethnomedically .

References

1. Adams, R.P., 1989 Identification of essential oil by ion trap mass spectroscopy. New York: Academic Press.
2. McLafferty, F. W. and Staufner, D.B. (1989). The Wiley NBS registry of mass spectral data. New York: John Wiley 302PP
3. Ansari. M.A. and Razdan, R. K. (1995). Repellent efficacy of various oils in repellingmosquitos. *Ind. J. Malariol.* **32**(3): 104-111.
4. Awuah, R. T. (1999). Inhibition of fungal colonization of stored peanut kernels with products from some medicinal/culinary plants. *Peanut Science* **29** (1): 13-17.
5. Der Marderosian, A. H. (2004). The reviews of natural products. Facts and comparison. St-Louis, Missouri. USA, 1000PP
6. Ikawa, S. (1918). Citronella oils. *J. Cem.Ind.Japan* **21**: 515-523
7. Kikino, H., Ogata, K., Kasahara, Y. and Konno, C. (1985). Pharmacology of ephedroxanes. *J. Ethnopharmacol.* **13**: 175-191.
8. Jennings, W. and Shibamoto, T. (1980). Quantitative analysis of flavour and fragrance volatile by glass capillary gas chromatography. New York: Academic Press.
9. Kondojoyan, N. and Berdague, J. L. (1996). A compilation of relative retention indices for the analysis of aromatic compounds. Ed. Laboratoire Flaveur, Saint Genes Champanelle, France, 234P.
10. Konwar, B. K. and Gohain, A. K. (1999). Nutritive value of spent citronella grass (*Cymbopogon nardus*) in cattle. *Ind. J. An. Nutr.* **16** (4): 324-325..
11. Koster, R., Anderson, M. and De Beer, E. .J. (1959). Acetic acid for analgesic screening. *Fed.Proc.* **18**: 412.
12. Lorke, D. (1983). A new approach to practical acute toxicity test. *Arch. Toxicol.* **54**: 275-287.
13. Moody, J. O. Adeleye, S. A., Guindidza, M. G., Wyllie, G. and Ajayi-Obé, O.O., (1995). Analysis of the essential oil of *Cymbopogon nardus* (L) Rendle growing in Zimbabwe. *Pharmazie* **50** (1): 74-75.

13. Manzoor-I-Khuda, M., Rahman, M., Yusuf, M. and Chowdhury, J. U. (1984). Essential oils of *Cymbopogon* species of Bangladesh. *J. Bangladesh Acad. Sci.* **8** (2): 77-80.
14. Nasada, Y. (1980). Analysis of essential oils by gas chromatography and mass spectrometry, John Wiley and Sons, Inc, vol 1 & 2, 133P.
15. Nature direct 2u-Essentials oils – Citronella – Cymbopogon nardus. <http://www.naturedirect2u.com/-Essential%20oils/citronella.htm>
16. Nigam, M. C., Datta, S. C. (1973). Recognition of authenticity of oil of *Cymbopogon* species by thin layer chromatography and gas liquid chromatography. *Parfuem. Kosmet.* **54** (11): 347-349.
17. Parimala Devi, B., Roominathan, R. and Mandal, S. C. (2003). Anti-inflammatory, analgesic and antipyretic properties of *Clitoria ternatea* root. *Fitoterapia* **74**: 345-349.
18. Ranaweera, S. S. and Dayananda, K.R. (1996). Mosquito-larvicidal activity of some Sri-Lankan plants *J. Nat. Sci. Council Sri Lanka* **24** (2): 63-69.
19. Von Sidow, E., Anjou, K. and Karlson, G., (1970). *Arch., Mass. Spectrography*. Academic press, San Diego, 302P
20. Zimmermann M. (1983). Ethical guidelines for investigation of experimental pain in conscious animals. *Pain* **16**: 109-110.