Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2014, 6(4):575-580

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Preliminary antihyperglycemic activity-guided studies on the leaf extract and fractions of *Ocimum basilicum* L.

F. N. Mbaoji*, C. O. Okoli and A. C. Ezike

Department of Pharmacology & Toxicology, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka, Enugu State, Nigeria

---

ABSTRACT

The methanol-dichloromethane leaf extract (MDE) of Ocimum basilicum L. (Lamiaceae)was subjected to biological activity-guided studies using antihyperglycemic effect in alloxan diabetic rats as activity-guide. The hypoglycemic and oral glucose tolerance effects were also evaluated in normal rats. The results showed that the extract and the fractions caused a significant ($P<0.05$) reduction in blood glucose level of alloxan diabetic rats. The level of reduction in blood glucose at 8 h was of the order of magnitude: MDE (Methanol-dichloromethane extract; 48.70%) > N-HF (N-Hexane fraction; 44.74%) > MF (Methanol fraction; 42.28%) > DF (Dichloromethane fraction; 31.88%). In normal rats, however, the extract did not reduce blood glucose level at doses tested but suppressed postprandial blood glucose rise after glucose load. Acute toxicity studies on the extract revealed an oral LD_{50}> 5 g/kg in mice. Preliminary phytochemical studies showed that the extract and fractions tested positive to carbohydrate, glycosides, reducing sugars, resins, saponins, steroids, and terpenoids. These findings suggest that constituents of leaves of O.basilicummay possess antihyperglycemic properties and lower blood glucose only in diabetic but not in normoglycemic condition. The antihyperglycemic activity may not be attributed to a single constituent.

Keywords: Alloxan, Antihyperglycemia, diabetic rats, *Ocimum basilicum* , Oral glucose tolerance.

INTRODUCTION

Current trends in the control of hyperglycemic condition associated with diabetes mellitus suggests roles for plants routinely used for culinary purposes. One of such plants is *Ocimum basilicum* L. (Lamiaceae), a perennial herb that grows from 30 - 90 cm high with opposite, light green, silky leaves ovate to lanceolate, 3.75 – 5 cm long and flowers 0.72- 1.25 cm long, borne on long terminal racemose inflorescence [1]. The plant is known by several vernacular names in Nigeria where it is variously called “Efirinpo”, “Efirin-ajija” and “Eirin-aja” in Yoruba, “esewon” in Edo, “ufuo-yibo” in Urhobo and “Urngo” and “Kacukacunga” in northern parts of the country [2,3].The leaves can be harvested throughout the growing season and are used fresh or dried for culinary and medicinal purposes. It is a culinary herb commonly used in the Mediterranean region [4], as well as in southeastern Nigeria where the leaves are popularly employed as flavoring in foods.

O. basilicum is additionally valued for its medicinal properties in traditional medicine where it is variously claimed to ease flatulence, stomach cramps, colic and indigestion following its actions on the digestive and nervous systems [5]. The leaves and flowering tops are credited with antispasmodic, carminative, digestive, galactogogue, stomachic and tonic [6,7,8,9] properties and claimed to be beneficial in the treatment of feverish illnesses (especially colds and influenza), nausea, gastro-enteritis, migraine, insomnia, depression and exhaustion when taken internally [10]. They are used to treat acne, insect stings, snake bites and skin infections [10] when applied topically. Infusion of the mucilaginous seed is used in the treatment of gonorrhoea, dysentery and chronic diarrhoea [11]. The plant is claimed to remove film and opacity from the eyes [9] whereas the root is used in the treatment of bowel complaints in

children [11]. Previous studies have revealed the antimicrobial [12], insecticidal [13], antihyperglycemic, hypolipidemic [14] and anticarcinogenic [15] activities.

The huge financial implication of diabetes management makes the search for alternative treatment from locally available medicinal or culinary plants an imperative. Although the antihyperglycemic activity of aqueous extract of this plant has been documented in an earlier study, this study subjected the methanol-dichloromethane leaf extract and its solvent fractions to bioactivity-guided studies using antihyperglycemic effect in alloxan diabetic rats as activity-guide. The hypoglycemic and postprandial glucose tolerance effects of the extract in normoglycemic rats were also studied.

EXPERIMENTAL SECTION

Animals

Adult Swiss albino rats (110-250 g) and mice (15-30 g) of both sexes were obtained from the Laboratory Animal Facility of the Department of Pharmacology & Toxicology, University of Nigeria, Nsukka. The animals were kept in steel cages within the Facility and allowed free access to water and standard livestock pellets. All animal experiments were conducted in compliance with the National Institute of Health Guidelines for Care and Use of Laboratory Animals (Pub No. 85 – 23, revised 1985).

Chemicals, reagents, solvents and drugs

All the chemicals, reagents and solvents used were of analytical grade. They include methanol (Fluka, England), dichloromethane (Fluka, England), n-hexane (NAAFCO, Nigeria), silica gel (60-120 mesh size), tween 80, alloxan (Fluka, Germany), glibenclamide (Aventis, Germany).

Equipment/instruments

Accu-Check® Active glucometer kit (Roche, Germany), Accu-Check® Active strips (Roche, Germany), Thomas-Willy Lab Mill (model 4), animal weighing balance, rotary evaporator, soxhlet extractor, bulk balance, fractionating column, animal cages.

Collectionand preparation of plant material

Fresh leaves of *O. basilicum* were purchased from a local market in Nsukka in November, 2009. The identity was established and authenticated at the International Centre for Ethnomedicine and Drug Development (InterCEDD), Nsukka. The leaves were separated from the stalk, dried under the sun for a day and ground to coarse powder using a laboratory mill (Thomas Willy, Model 4). The leaf powder was weighed and stored in an air tight container before use.

Extraction of plant material and solvent-guided fractionation of extract

About 960 g of the powdered leaves was extracted with about 5 L of a 1:1 mixture of methanol-dichloromethane by continuous extraction in a soxhlet. The extract was concentrated in a rotary evaporator to obtain 87.13 g (9.07% w/w) of the methanol-dichloromethane extract (MDE). Subsequently, MDE (56.17 g) was subjected to solvent guided fractionation in a silica gel (60 – 120 mesh size) glass column (60 x 4.5 cm) successively eluted with n-hexane, dichloromethane, and methanol (100%). The fractions were collected and concentrated in a rotary evaporator to afford 11 g (19.58% w/w) of n-hexane (N-HF), 12.6 g (22.43% w/w) of dichloromethane (DF) and 12.8 g (22.78% w/w) of methanol (MF) fractions respectively. The extract and fractions were subjected to preliminary phytochemical tests using procedures outlined by [16], [17]

Acute toxicity (LD_{50}) test

The acute toxicity and lethality (LD_{50}) of the methanol-dichloromethane extract (MDE) in mice (n = 13) was estimated using the method described by [18]. The study was carried out in two stages. In stage one, mice (n = 3) received oral administration of 10, 100, or 1000 mg/kg of MDE (suspended in3% Tween 80) and were observed for 24 h for number of deaths. At the end of 24 h, no death was recorded. Consequently, a fresh batch of mice (n = 1) received 1600, 2900, and 5000 mg/kg of MDE in the second stage of the test and were observed for 24 h for deaths.

Biological activity-guided studies

The extract and fractions were subjected to bioactivity-guided studies using antihyperglycemic activity in alloxan-induced diabetic rats as activity guide. The animals were fasted for 12 h but allowed free access to water. At the end of the fasting period, the basal fasting blood glucose (FBG) level was measured using a glucometer. Subsequently, diabetes was induced by single intraperitoneal injection of alloxan monohydrate (120 mg/kg) and normal feeding maintained thereafter. Five days later, blood was drawn from each rat by tail snipping and the blood glucose level measured using Accu-Check® Active glucometer kit. Animals with blood glucose level ≥ 170 mg/dl were

considered diabetic and used for the study. The diabetic animals were randomly divided into 10 groups (n = 5) and received oral administration of 200 or 400 mg/kg of MDE, N-HF, DF, MF, 3% v/v Tween 80 (vehicle) (2 ml/kg) or glibenclamide (0.2 mg/kg) respectively. Blood glucose was measured as earlier described before (0 h) and at 0.5, 1, 2, 4 and 8 h after treatment. The results showed that the MDE elicited greater hypoglycemic effect than the fractions. Hence the fractions were not subjected to further studies.

Hypoglycemic activity test

Animals fasted for 16 h, were randomly divided into four groups (n = 5) and received oral administration of MDE (200 and 400 mg/kg), glibenclamide (0.2 mg/kg) and 3% v/v Tween 80 (2 ml/kg) respectively. The blood glucose level of each animal was measured prior to (0 h) and at 0.5, 1, 2, and 4 h after treatment.

Oral glucose tolerance test

Animals fasted for 16 h but with free access to water, were randomly divided into four groups (n = 5) and received oral administration of MDE (200 and 400 mg/kg), glibenclamide (0.2 mg/kg) and 3%v/v Tween 80 (2 ml/kg) respectively. One hour later, the rats received oral glucose load (2 g/kg). The blood glucose level of animals in each group was measured before (0 h) and at 30, 60, 90, 120,150 and 180 min after glucose load.

Statistical analysis

Data obtained was analyzed using One-Way ANOVA and further subjected to LSD test post hoc and presented as Mean ± SEM. Differences between means were accepted significant at 95% confidence interval.

RESULTS

Acute toxicity test

Oral administration of up to 5 g/kg of MDE to mice did not cause any death. The oral LD_{50} of MDE in mice was estimated to be > 5 g/kg.

Phytochemical constituents of extract and fractions

The phytochemical tests showed that the MDE tested positive to glycosides, reducing sugars, steroids, saponins, terpenoids, carbohydrates and resins. The N-HF tested positive to glycosides, reducing sugars, steroids and terpenoids, while the DF gave positive reactions for glycosides, carbohydrates, reducing sugars, steroids and terpenoids. The MF tested positive to glycosides, saponins, carbohydrates reducing sugars and resins (Table 1).

Table 1: Phytochemical constituents of extract and fractions

Phytoconstituent	MDE	N-HF	DF	MF
Alkaloids	-	-	-	-
Carbohydrates	++	-	+	++
Flavonoids	-	-	-	-
Glycosides	++	+	++	++
Reducing sugars	++	+	++	+
Resins	+++	-	-	++
Saponins	+++	-	-	+++
Steroids	++	++	++	-
Tannins	-	-	-	-
Terpenoids	+++	+++	++	-

- = Absent; + = Present in small concentration; ++ = Present in moderately high concentration; +++ = Present in very high concentration; MDE = Methanol-Dichloromethane extract; N-HF = N-hexane fraction; DF = Dichloromethane fraction; MF = Methanol fraction

Effect of extract and fractions on blood glucose level of diabetic rats

The extract and fractions caused significant (P<0.05) reduction in the blood glucose level of diabetic rats in a non-dose dependent manner (Table 2). There was an initial increase in the blood glucose level upon administration of the drugs followed by a non-uniform decrease over time. MDE, N-HF, MF, and glibenclamide reduced blood glucose level from 2-8 h while DF at 200 mg/kg reduced blood glucose level from 4-8 h whereas the 400 mg/kg elicited no reduction. The antihyperglycemic effect was of the order of magnitude MDE >HF>MF>DF (Table 2).

Effect of MDE on blood glucose level in normoglycemic rats

Acute oral administration of MDE to normoglycemic rats did not lower blood glucose level. However, there was a decrease in the blood glucose level of glibenclamide treated rats (Table 3).

Effect of MDE on oral glucose tolerance

The extract suppressed the postprandial rise in glucose level in a non-dose-dependent manner after the glucose meal. Peak suppression occurred at 30 – 60 min after the glucose load (Table 4).

Table 2: Effect of extract and fractions on blood glucose level of diabetic rats

Treatment	Dose (mg/kg)	Blood glucose level (mg/dL)					
		Baseline	0.5 h	1 h	2 h	4 h	8 h
Control	-	447.2± 43.0	477.2± 34.7 (-6.71)	484.4 ± 33.0 (-8.31)	497.8± 33.4 (-11.31)	487.0± 36.5 (-8.90)	487.2± 46.5 (-8.94)
MDE	200	321.6± 53.7	338.6 ± 46.7 (-5.29)	325.4 ± 35.6 (-1.18)	316.2 ± 40.5 (1.68)	274.2 ± 41.7 (14.74)	292.8 ± 50.8 (8.96)
	400	324.4± 50.5	350.2 ± 41.3 (-7.53)	339.8 ± 33.1 (-4.75)	326.2 ± 34.0 (-0.55)	167.2 ± 53.6* (48.46)	166.4 ± 33.3* (48.71)
N-HF	200	222.6± 23.4	257.8 ± 36.5 (-15.81)	259.0 ±35.7 (-16.35)	228.0 ± 45.1 (-2.43)	144.0 ± 25.7 (35.31)	123.8 ± 21.5* (44.38)
	400	245.2± 44.3	283.0 ± 32.8 (-15.42)	265.0 ± 37.2 (-8.08)	233.2 ± 40.4 (4.90)	180.8 ± 45.9 (26.26)	161.0 ± 47.0 (34.34)
DF	200	193.2± 36.0	236.8 ± 31.2 (-22.57)	227.0 ± 28.2 (-17.49)	194.2 ± 30.7 (-0.52)	165.0 ± 28.2 (14.60)	131.6 ± 30.8 (31.89)
	400	257.0±3 0.8	339.4 ± 33.8 (-32.06)	323.2 ± 36.8 (-25.76)	344.8 ± 61.9 (-34.16)	323.2 ± 82.2 (-25.76)	298.2 ± 101.8 (-16.03)
MF	200	260.6± 44.9	289.0 ± 26.6 (-10.90)	299.8 ± 29.5 (-15.04)	229.8 ± 25.9 (11.82)	202.6 ± 16.3 (22.56)	150.4 ± 23.1* (42.29)
	400	310.2± 36.0	352.2±47.3 (-13.54)	377.2 ± 59.7 (-21.60)	323.4 ± 60.1 (-4.26)	283.8 ± 72.0 (8.51)	256.8 ± 82.2 (17.21)
Glibenclamide	0.2	280.0 ±30.4	299.4 ± 19.7 (-6.93)	302.8 ± 23.2 (-8.14)	264.6 ± 14.4 (5.50)	214.0 ± 22.0 (23.57)	181.6 ± 23.8* (35.14)

*n = 5; *P<0.05 compared tobaselinevalues(One way ANOVA; LSD test post hoc);MDE – Methanol-Dichloromethane extract; N-HF - N-hexane fraction; DF - Dichloromethane fraction; MF - Methanol fraction. The values in parenthesis represent percentage reduction of blood glucose calculated relative to baseline glucose level.*

Table 3: Effect of MDE on blood glucose concentration in normoglycemic rats

Treatment	Dose (mg/kg)	Blood glucose (mg/dL)				
		0 h	0.5 h	1 h	2 h	4 h
Control	-	115.8 ± 2.5	123.4 ± 2.0	123.4 ± 2.0	114.0 ± 1.8	113.8 ± 3.2
MDE	200	103.4 ± 7.5	116.2 ± 6.2	119.8 ± 5.5	118.2 ± 3.7	112.2 ± 5.2
	400	111.6 ± 4.7	132.8 ± 5.4	124.0± 4.9	116.8 ± 3.1	124.0 ± 3.8
Glibenclamide	0.2	154.0 ± 4.8	116.2 ± 3.1	118.6 ± 2.7	107.2 ± 2.7	93.4 ± 2.6

MDE: Methanol-dichloromethane extract

Table 4: Effect of MDE on postprandial blood glucose level in normoglycemic rats

Treatment	Dose (mg/kg)	Blood glucose (mg/dL)						
		0 min	30 min	60 min	90 min	120 min	150 min	180 min
Control	-	117.4 ±2.8	169.2±19.5 (44.12)	164.2±19.2 (39.86)	132.4±9.2 (12.78)	131.8±9.8 (12.27)	128.2±9.9 (9.20)	129.2±7.9 (10.05)
MDE	200	124.2± 4.3	143.6±9.1 (15.62)	132.2±5.3 (6.44)	132.0±3.9 (6.28)	128.6±4.9 (3.54)	127.2±3.7 (2.43)	126.6±3.2 (1.93)
	400	129.0 ±6.5	139.6±8.7 (8.22)	144.4±10.3 (11.94)	131.4±7.1 (1.86)	129.6±3.9 (0.47)	126.6±5.6 (-0.19)	122.2±6.2 (-0.53)
Glibenclamide	0.2	103.2 ±3.2	141.4±12.3 (37.02)	120.4±7.7 (16.67)	109.4±6.5 (6.01)	104.4±7.1 (1.16)	95.8±10.1 (-7.17)	88.2±5.3 (-14.54)

Values in parenthesis are glycemic change (%) calculated relative to 0 min values. MDE = Methanol-dichloromethane extract.

DISCUSSION

Results from this study have shown that the extract and fractions of *O. basilicum* leaves may lower blood glucose level in diabetic but not in normoglycemic condition. Experimentally, alloxan is known to cause hyperglycemia and glucose intolerance or syndromes similar to either Type 1 or Type 2 diabetes mellitus [19,20] Therefore, effective lowering of blood glucose levels in treated diabetic rats by the extract and fractions indicates that leaves of this plant may be useful in overt cases of diabetes mellitus. However, results of this study also showed that the glycemic control ability of constituents of the leaves may be restricted to diabetic conditions since treatment of normoglycemic rats with the extract and fractions did not lower their blood glucose levels. This is rather not surprising since the leaves are popularly used as flavoring in food and there is no known or documented association of its use with accidental hypoglycemia after consumption. Thus, in addition to the likely usefulness of the leaves in overt diabetes condition, our findings suggest that culinary use of the leaves may not predispose normal individuals to the risk of hypoglycemia.

Equally important is the effect of the extract on glucose tolerance. Pre-treatment of normal rats with the extract suppressed postprandial rise in blood glucose level rise after a heavy glucose meal with maximum suppressive effect coinciding with the time (30 - 60 min) of peak blood glucose level after the meal. This effect suggests that the extract may be effective in controlling overt postprandial rise in blood glucose, a common feature known to

aggravate the risk of chronic hyperglycemia in diabetes mellitus. Thus, control of postprandial hyperglycemia in diabetes is of great importance due to its close relation to the risk of micro and macro-vascular complications and death [21, 22]. A combination of antihyperglycemic and enhanced glucose tolerance effects makes the leaves of this plant attractive in glycemic control in diabetes.

Solvent-guided fractionation was employed to simplify the complexity of the extract mixture as a prelude to identifying the antihyperglycemic constituent or constituents using bioactivity-guided technique. Comparison of the magnitude of reduction in blood glucose level of diabetic rats in the activity-guided study showed that the extract, MDE, was more effective than the most active fraction. This implies that the antihyperglycemic activity may not increase with further fractionation or separation of constituents of MDE. As such, the antihyperglycemic activity may not be ascribed to a single constituent. It is thus likely that leaf extracts of *O. basilicum*may derive its antihyperglycemic effect largely from the combined effects of the phytoconstituents. This notwithstanding, further separation of the most active fraction may yield antihyperglycemic constituents with albeit a lower potency than the extract. Several studies have documented the antihyperglycemic activity of phytoconstituents isolated from different plants. Some of these phytoconstituents include the glycosides- isoorientinin from *Cecropiaobtusfolia*[23] and triterpenoids, such as ursolic acid from *Fructuscoini* [24]; the sesquiterpenoid, β-Eudesmol from *Rhizomaatractylodis*[25] as well as polysaccharides from *Radix ophropogonis*[26, 27]. These phytoconstituents have all demonstrated antihyperglycemic activity.

Oral administration of the extract to mice did not cause death at doses ranging from 10-5000 mg/kg, suggesting high degree of relative safety from acute toxicity. This high degree of relative safety may be restricted only to acute intoxication since *O. basilicum* was documented to contain estragole – a known carcinogen in rodents [28]. The extent of occurrence of this constituen

t in the extract and fractions used in this study was not evaluated. Therefore, the plant leaves may not be generally regarded as safe.

CONCLUSION

Results of this study have shown that constituents of leaves of *O.basilicum* may possess antihyperglycemic properties and lower blood glucose only in diabetic but not in normoglycemic condition. The antihyperglycemic activity may be attributed in large part to the combined effects of constituents of the leaves. Further studies to isolate antihyperglycemic constituents and elucidate the mechanisms of blood glucose lowering effect in diabetes are encouraged.

Acknowledgement
The authors thankfully acknowledge the financial assistance from the Nigeria-Sao Tome and Principe Joint Development Authority for their 2009 Scholarship Award and the Federal Ministry of Education, Nigeria Science and Technology Education Post Basic (Step b) Project (cr: 4304-uni) for 2010 *Innovators of Tomorrow Research and Technology Development Grant* award both to F. N. Mbaoji.

REFERENCES

[1] PP Joy;J Thomas; S Mathew; G Jose; J Joseph. *Aromatic plants. Tropical Horticulture* Vol. 2. (Eds. Bose, T.K., Kabir, J., Das, P. and Joy, P.P.). Naya Prokash, Calcutta, **2001**, 633-733.
[2] BC Ndukwu; NB Ben-Nwadibia. *Ethnobotanical Leaflets*. **2005**, 2005(1),10.
[3] R Blench; M Dendo. FulfuldeNames for Plants and Trees in Nigeria, Cameroun, Chad and Niger. **2006**; http://www.rogerblench.info/Ethnoscience%20data/Fulfulde%20Plant%20names.pdf
[4] MA Grieve. Modern Herbal.Vol 1. New York: Hafner,**1967**, 86, 2.
[5] A Chevallier. TheEncyclopediaofMedicinalPlants Dorling Kindersley. London **1996**; ISBN 9-780751-303148.
[6] J Holtom; W Hylton. CompleteGuidetoHerbs.Rodale Press, Pennisylvania, USA.ISBN 0-87857-262-7.**1979**.
[7] J Lust. TheHerbBook. Bantam books, USA. ISBN 0-553-23827-2.**1983**.
[8] R Chiej**.**EncyclopaediaofMedicinalPlants.MacDonald **1984**; ISBN 0-356-10541-5.
[9] JA Duke; ES Ayensu.MedicinalPlantsofChina Reference Publications, Inc.**1985**ISBN 0-917256-20-4.
[10] D Bown**.**EncyclopaediaofHerbsandtheirUses. Dorling Kindersley, London. **1995**; ISBN 0-7513-020-31.
[11] RN Chopra; SL Nayar; IC Chopra.Glossary of Indian Medicinal Plants (Including the Supplement).**1986**; Council of Scientific and Industrial Research, New Delhi.
[12] B Wannissorn; S Jarikasem; T Siriwangchai; S Thubthimthed. *Fitoterapia*.**2005**, 76, 233-236.
[13] R Pavela. *Fitoterapia*. **2004,** 75,745-749.
[14] NA Zeggwagh; T Sulpice; M Eddouks. *Am. J.Pharmacol Toxicol.*,**2007**, 2(3), 123-129.
[15] T Dasgupta; AR Rao; PK Yadava. *Phytomedicine*.**2004**, 11, 139-151.

[16] JB Harborne. The Flavonoids. In: Advances in Research, Chapman and Hall, London. **1973**.
[17] WC Evans. Trease and Evans Pharmacognosy. W.B Saunders & Co, London **1989**, 33.
[18] D Lorke. *Arch Toxicol.*,**1983**,54, 275- 281.
[19] S Lenzen; M Tiedge; AJorns; R Munday. Alloxan derivatives as a tool for the elucidation of the mechanism of the diabetogenic action of alloxan. In: (Shafrir, E. (ed.)) Lessons from Animal Diabetes, Birkhauser, Boston, **1996**; 113-122.
[20] TS Fröde; YS Medeiros. *J. Ethnopharmacol*. **2008**, 115, 173-183.
[21] B Balkau. *Diabetes Metab*.,**2000**, 26, 282-286.
[22] A Ceriello. *Diabetes*.**2005**, 54, 1-7.
[23] A Andrade-Cetto; H Wiedenfeid. *J. Ethnopharmacol*.,**2001**, 78(2-3), 145-9.
[24] J Yamahara; H Mibu; T Sawada; H Fujinura; S Takino; M Yoshikawa; I Kitagawa. *YakugakuZasshi*.,**1981**, 101, 86-90.
[25] M Kimura; PV Diwan; S Yanagi; Y Kon-no; H Nojima; I Kimura. *BiolPharm Bull.*, **1995**,18, 407-410.
[26] WX Zhang; NH Wang. *Chinese Trad Herbal Drugs*.,**1993 a,** 24, 30-31.
[27] WX Zhang; NH Wang. *Chinese Trad Herbal Drugs*.,**1993 b**, 24, 30-31.
[28] European Agency for the Evaluation of Medicinal Products (EMEA), Working Party on Herbal Medicinal Products (HMPWP).Final Position Paper on the Use of Herbal Medicinal Products Containing Estragole. London: EMEA. **2004** Retrieved April 4, 2008.