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EFFECTS OF GRADED LEVELS OF *DIOSCOREA VILLOSA* EXTRACTS ON PHYSIOLOGICAL RESPONSE OF WEST AFRICAN DWARF GOAT.

[1]Okukpe K. M*., [1]Adeloye A. A., [2]Soladoye A. O., [3]Adua M. M., [1]Aderibigbe T.A. and [1]Opowoye I. O.
[1]Reproductive Physiology Unit, Department of Animal Production, Faculty of Agriculture, University of Ilorin; [2]Department of Physiology, College of Medicine, University of Ilorin, P.M.B. 1515, Ilorin, Kwara State, Nigeria; [3]Department of Animal Science, Nasarawa State University, Keffi, Nasarawa State, Nigeria.

Corresponding author: - okukpekehinde@yahoo.com

Thirty (30) West African dwarf bucks aged 12 to 24months were used in an experimental design to assess the effect of *Dioscorea villosa* extract on the sexual respone of the West African Dwarf bucks. They were subcutaneously administered 0.01, 0.02, 0.03ml/kg body weights of *Dioscorea villosa* extract. The control and standard group were given 0.01ml/kg body weights of normal saline and sildenafil citrate solution respectively.
The study indicates that subcutaneous administration of 0.01ml/kg of *Dioscorea villosa* increase sexual drive (libido) in WAD bucks. Animals in Group C, treated with 0.01ml/kg of *Dioscorea villosa* extract showed signs of active sexual activity (aggressiveness) and mounting which reduce with additional increase in extract administration. There was also significant improvement in sperm count at this dosage. This corresponds with the decreasing serum testosterone level as the extract dose was increased. Thus, the aphrodisiac activity of the extract might be attributed to its balancing of endogenous hormone in the animal and enhancement of the haemopoietic system. Phytochemical studies indicate the presence of high levels of saponin, alkaloids and minimal flavonoid along with oxalate and phytates in the extract. Hence, the sexual function improving effect of the extract might be due to the presence of such compounds.

Keywords: Dioscorea villosa, extracts, West-African Dwarf goats, Libido, Subcutaneous.

Traditional medicine is practiced in different parts of the world, especially among the low income earners. Despite the wide acceptance of plants as source of medicaments by many, only very few of these herbs have been properly identified and documented (Akinniyi *et al.*, 1986). Few herbal plants have found their use in reproductive management of animals (Tajudeen *et al.*, 2005; Gauthaman *et al.*, 2008 and Okukpe *et al.*, 2012a, b).

Dioscorea villosa or wild yam is an herbaceous twinning perennial which belongs to the family Dioscoraceae. Common in China mainland, Mexico, east/ central United State as well as tropical African countries. Twinning in hedges, over bushes and fences, the slender tuberous root stock is crooked and laterally branched; the leaves are broadly ovate and cordate, glabrous on top and finely hairy underneath. The rhizomes are slightly cylindrical curved and slightly flattened, hard and solid with white/ starchy surface when broken. Taste mild and slightly sour, sticky when chewed. Its contraceptive use is attributed to the action of various steroidal saponins(diosgenin and aglycone) and also to dioscorine, dioscine and other alkaloids derived from nicotinic acid. The root also contains phytosterols, alkaloids, tannins and a high level of starch (Watson, 1993; Wren, 1994). It also shows antibacterial activity (Kelmanson, 2002). It has a long history of being used in the treatment of an assortment of female health conditions such as hot flashes, menstrual-cramps, vaginal dryness, premenstrual syndrome, curing morning sickness among pregnant women and to

alleviate pains endured during child-birth (Brinker, 1997; Mckee, 2008). Its contraceptive use is attributed to the action of various steroidal saponins (diosgenin and aglycone) and also to dioscorine, dioscine and other alkaloids derived from nicotinic acid. Diosgenin is structurally close to the female hormone, progesterone and has been used to manufacture the first ever oral contraceptive to prevent unwanted pregnancy. In addition, extracts from *D. villosa* has been used as an alternate for oestrogen cream that facilitates neutralizing vaginal dryness in women (Brinker, 1997; Moerman, 1998; Cech, 2000). Marker (1940) described methods for conversion of diosgenin into testosterone and other steroids. Six saponins have been reported for *D. villosa*: protodioscin, ME protodioscin, parrisaponin, dioscin, progenin III (prosapogenin A of dioscin) and progenin II (Sautour *et al*., 2006; 2007; Hayes *et al*., 2007; Hu *et al*., 2007). The major saponin activity is that of dioscin and diosgenin. Despite the fact that the molecule can be converted to hormones such as progesterone and dehydroepiandrosterone(DHEA) by several enzymatic steps in the laboratory, there is conflicting attempts to market it as such have been dubbed by some as "the wild yam scam"(Higdon *et al*., 2000; Foster and Johnson, 2006). However, oestrogenic action on the mammary epithelium of ovariectomized mice has been reported (Aradhana *et al*., 1992) while other studies have provided mixed results (Zava *et al*., 1998). Diosgenin was reported to protect rat kidneys from morphological changes associated with ovariectomy, posited as occurring due to the conversion of diosgenin to progesterone in- vivo (Tucci and Benghuzzi, 2003). However, any direct hormonal effect that could be attributed to *D. villosa* is reported to be oestrogenic (Morgan, 2011). Administration of diosgenin containing edible yam (*D. alata*) to postmenopausal women as 30% of their diet for 30days was reported to cause increase in serum oestrogen with a concurrent reduction in serum androgen levels(Wu *et al.,* 2005).

The physiological enhancing and reproductive health assisting capability of the above plant is the thrust of this study. This study reports the effect of Dioscorea *villosa extracts* on the physiological well-being of the West African dwarf buck.

MATERIALS AND METHODS

Preparation of extract

Dioscorea villosa rhizomes were gotten within Ilorin metropolis. It was cleaned to remove dirt, air-dried, pound to pulp and packaged in cellophane bag for extraction. The extraction of the active substance of the plant parts were carried out with a modified method of Swain (Swain, 1999) with 96% ethanol at 75- 80^{o}C in a soxhlet apparatus for four hours. At the end of the extraction, the liquid extracts were filtered using Whatman filter paper. The filtrate were concentrated under reduced pressure in a vacuum dessicator at 30^{o}C for 25minutes using a rotary evaporator (Gallenkamp, UK) to obtain a dark brown mass. The resulting residues (extract) were transferred to a hot air oven where they were dried to a constant weight at 45^{o}C. Subsequently, 5g of the extract was dissolved in 250ml of saline water to obtain appropriate concentrations suitable to achieve a 0.5ml dose by volume of extract which was later administered subcutaneously to the animals for a period of seven days.

Management of animals

Thirty healthy male West African dwarf goats aged 12 to 24months were used for this experiment. The animals were quarantined for fourteen days during which they were treated against PPR using Tissue Culture Rinderpest Vaccine and dewormed using Albendazole at a dosage of 2ml/10kg body weight. They were examined to be free of any obvious abnormalities of the palpable reproductive organs.

Housing: They were housed in pens with slatted wooden floors with wooden feeding trough and plastic drinker. Animals were randomly assigned to five treatment groups of six animals each after equalization of weight.

Feeding: The animals were managed intensively. They were fed *Panicum*

maximum ad- libitum and 500g/animal of concentrate ration consisting of wheat offal 40 %, corn offal 35% , palm kernel cake 22%, bone meal 2%, mineral / salt mixture 1 % to give a crude protein of 17.20, crude fibre 12.11, ether extract 3.63 and ash 9.35. Water was provided *ad-libitum*. They were weighed, their heart beat and pulse rate as well as rectal temperature were taken and recorded weekly for the period of seven days the experiment lasted. Feed and water were provided *ad-libitum*.

Experimental procedure

The goats were randomly assigned into five treatments of six animals each. Ethanolic extract of *Dioscorea villosa* (0.01ml/kg, 0.02ml/kg, 0.03ml/kg body weight) were administered subcutaneously to treatments C, D and E respectively while A served as the control, B served as standard and received 0.01ml/kg normal saline and sildenafil citrate respectively. Blood was collected on the third and sixth day of administration of extracts from each animal into heparinized and plain sample bottles for the determination of complete blood count and serum chemistry respectively.

Haematological and Biochemical assay

The haematocrit, blood counts, total protein and albumin were determined by standard methods.

Statistical analysis

Data on physiological indices (blood-pressure, pulse-rate, rectal temperature) as well as, reproductive (sperm count, live-dead ratio, sperm motility, abnormality), haematology (PCV, Hb, RBC, WBC) and biochemical parameters (TP and ALB) were analyzed using the analysis of variance (ANOVA) procedure following a completely randomized model (Steel and Torrie, 1990) and the level of significance were determined using the Duncan's Multiple Range Test (Duncan, 1955).

RESULTS AND DISCUSSION

The physiological, haematological and biochemical effect of *Dioscorea villosa* on West African Dwarf bucks are shown in Table 1. The blood-pressure, pulse rate and temperature all followed the same trend and were highest in group C, followed by D. It was observed that Hb, PCV, RBC and WBC increase with the level of extract and was significantly different ($p < 0.05$). There were no significant differences in serum total protein (TP) between groups C, D and E but differ significantly when compared to groups A and B. TP increase as the level of extract is increased, but reduces at the highest dosage of 0.03ml/kg body weight. The serum albumin levels of groups A, B, C and E were not significantly different($p > 0.05$) but differ significantly from group D, the highest was observed in group E and the lowest was in group D. The results of this study show that the ethanolic extract of *Dioscorea villosa* rhizome administered subcutaneously at the dosages used for the period of experiment appears to enhance the haemopoietic system. The increase may have occurred due to reduction in the destruction of blood cells and probably increased blood cell synthesis (Duncan *et al.*, 1994; Son *et al.*, 2007; Ilesanmi, 2009). This could also be observed in the increased total serum protein and albumin as extract doses increase. This seems to support previous hypothesis that *Dioscorea villosa* and other *Dioscorea* species possesses dehydroepiandrosterone (DHEA)- like properties and acts as a precursor to human sex hormones such as estrogen and progesterone, hence its use in the treatment of painful menstruation, hot flashes and headaches associated with menopause(Wren, 1994; Scott *et al.*, 2000; Eagon *et al.*, 2001; Komesaroff *et al.*, 2009). The effect of *Dioscorea villosa* saponin constituent diosgenin on lipid metabolism are well documented and are probably due to impaired intestinal cholesterol absorption (Foot and Duke, 1990; Watson, 1993; Son *et al*., 2007). There have been studies to show that Dioscorea has antioxidant activities (Araghiniknam *et* al., 1996; Middleton *et al.*, 2000; Oda *et al.*, 2000; Raju *et al.*, 2007), and the anti-inflammatory activity can be linked to the anti-phlegistic effect of the steroidal saponins. This could be linked to the increased serum total protein (TP) when compared with the control as well as the globulin level since albumin was not significantly different ($p > 0.05$). Some

Table 1. Effect of *dioscorea villosa* (dv) on haematological, biochemical and physiological parameters of west african dwarf bucks.

Parameters	A 0.01ml of saline	B 0.1ml of Sc	C 0.01ml of Dv extract	D 0.02ml of Dv extract	E 0.03ml of Dv extract	SEM
Hb , g/dl	6.10^{b}	8.10^{a}	8.10^{a}	8.70^{a}	7.90^{a}	±0.048
PCV, %	18.00^{c}	24.00^{b}	29.00^{a}	30.00^{a}	25.10^{b}	±2.638
RBC, $\times 10^{6}$ mm^{3}	2.10^{b}	3.10^{a}	3.00^{a}	3.40^{a}	2.30^{b}	±0.065
WBC, $\times 10^{3}$ mm^{3}	6.80^{c}	8.20^{b}	9.40^{b}	10.20^{a}	10.60^{a}	±0.058
Total protein, g/dl	53.00^{c}	62.00^{b}	67.00^{a}	72.00^{a}	68.00^{a}	±3.523
Albumin, g/dl	36.00^{a}	35.00^{a}	36.00^{a}	32.60^{b}	37.00^{a}	±5.503
Blood pressure, mmHg	$118^{b}/69^{b}$	$102^{c}/68^{b}$	$134^{a}/111^{a}$	$131^{a}/181^{a}$	$106^{b}/82^{b}$	±14.00/ 9.53
Pulse-rate, beat/min.	68.00^{b}	78.30^{a}	76.50^{a}	71.00^{b}	73.40^{ab}	± 9.27
Rectal temp., ^{O}C	38.20	38.40	38.70	38.70	38.50	±0.08

Means with different superscript on the same row are significantly different (P< 0.05). Dv- Dioscorea villosa, *Sc- Sildenafil- citrate, BW- Bodyweight,* SEM- Standard error of the mean, PCV- Packed cell volume, RBC- Red blood cell, Hb- Haemoglobin, WBC- White blood cell.

Table 2. Effect of dioscorea villosa (dv) on reproductive function of west african dwarf bucks.

Parameters	A 0.1ml of saline	B 0.1ml of Sc	C 0.1ml of Dv extract	D 0.2ml of Dv extract	E 0.3ml of Dv extract	SEM
Sperm count , 10^{9}/ml	1.38^{bd}	1.65^{acd}	1.90^{a}	1.44^{bd}	1.30^{bc}	±0.13
Motility, %	65.00^{a}	40.50^{b}	45.30^{b}	31.20^{c}	14.70^{d}	±2.15
Live-dead ratio	4.53^{ac}	3.16^{bc}	5.47^{a}	1.96^{b}	1.65^{b}	±0.65
Semen pH	6.90	7.10	6.80	7.30	7.10	±0.27
Live spermatozoa, %	81.50^{a}	75.30^{ac}	83.46^{a}	64.60^{bc}	61.30^{b}	±3.81
Sperm abnormality, %	7.33^{b}	12.33^{a}	9.62^{b}	16.23^{a}	14.75^{a}	±1.22
Serum testosterone, g/dl	1.20	1.40	1.40	1.20	1.30	±0.12

Means with different superscript on the same row are significantly different (P< 0.05). Dv- Dioscorea villosa, *Sc- Sildenafil- citrate,* SEM- Standard error of the mean.

species of *Dioscorea* (*D. sylvatica* and *D. dregeana*) have been reported to show antibacterial activity against Gram-positive bacteria and Gram-negative, *Escherichia coli* (Kelmanson, 2002). The significant improvement in sperm count (Table 2) at the lowest dose of 0.01ml/kg BW lend support to its use as sexual stimulant or aphrodisiac either singularly or in combination with other herbal plants in human (Qureshi *et al.*,

1989; Liu, 2004; Evan, 2009). This could be due to its possession of oestrogen-like property (Yen *et al.*, 2005; Wu *et al.*, 2005 and Morgan, 2011). All the other reproductive parameters studied reduced with increasing extract doses except in 0.01ml/kg BW. This support previous work that *Dioscorea villosa* has oestrogenic activity (Brinker, 1997; Aradhana *et al.*, 1992 and Morgan, 2011). It has been reported to increase serum oestrogen and decrease serum androgen or testosterone levels (Wu *et al.*, 2005) as was observed in the decreasing serum testosterone level as the extract doses was increased in this research. The reduced effect in all the parameters studied at doses higher than 0.01ml/kg may likely be as a result of negative effect on the animals' well-being, supporting the reports of Wojcikowski *et al.* (2008) that it causes chronic kidney injury when used for long period and at high doses.

CONCLUSIONS

In conclusion, *Dioscorea villosa* rhizome extract at a dose of 0.01ml/kg body weight support haemopoietic system by helping to improve blood synthesis or reduce its destruction. It could provide balance for endogenous sex hormone in terms of an increase in serum oestrogen while at the same time decreasing serum androgen. When used in combination with other androgen enhancing extracts it could prevent an abrupt increase in serum androgen and helps to reduce withdrawal symptoms associated with androgen usage in human.

REFERENCES

1. Akinniyi J.A., Sultanbawa M.U.A. and Manawaku D. (1986). Ethnobotany and Ethnopharmacology of Nigerian Medicinal plants. In the state of Medicinal plant Research in Nigerian. Ibadan. University press, p154-165.
2. Aradhana Rao A.R. and Kale R.K. (1992). Diosgenin - a growth stimulator of mammary gland of ovariectomized mouse. Indian Journal of Experimental Biology 30(5): 367-70.
3. Araghiniknam M., Chung S., Nelson-White T., Eskelson C. and Watson R.R. (1996). Antioxidant activity of Dioscorea and dehydroepiandrosterone (DHEA) in older humans. Life Sciences 59(11): PL147- PL157.
4. Brinker F. (1997). Herbal Contraindication and Drug interaction: Plus Herbal Adjuncts with Medicines, 4th ed. Ecletic Medical Publication.
5. Brinker F. (2008). Wild yam – sorting out the species. J American Herbalists Guild 8 (2), pp. 3-13.
6. Cech R. (2002). Growing At-risk Medicinal Herbs: Cultivation, Conservation, and Ecology. Williams, OR: Horizon Herbs. www-personal.umich.edu/~rburnham/SpeciesAccountspdfs/DiosvillDIOSFINAL.pdf .
7. Duncan D.B. (1955). Multiple ranges and multiple F – test. Biometrics II: 1- 42.
8. Duncan J.R., Prasse K.W. and Mahaffey E.A. (1994). Veterinary Laboratory Medicine (Clinical Pathology), Iowa State University Press.
9. Eagon P.K., Elm M.S, Hunter D.S. (2000). Medicinal Herbs: Modulation of Oestrogen action. Era of Hope Mtg, Dept Defense; Breast Cancer Res Prog, Atlanta, GA, June 8-11.
10. Evans W.C. (2009). Trease and Evans Pharmacognosy, 16th edition, Saunders Elsevier Ltd pub. Edinburgh, page 305.
11. Foster S. and Duke James A. (1990). Eastern/Central Medicinal Plants. Peterson Field Guides. ISBN No. 0-395-35309-2 also 0-395-46722-5 (pbk). Houghton Mifflin.
12. Foster S. and Johnson R.L. (2006). Desk reference to nature's medicine.Washington D.C.: National Geographic.
13. Gauthaman K., Ganisan A.P. and Prasad R.N. (2008). Sexual effects of puncture vine (Tribulus terrestris) extract (protodioscin) and evaluation using a rat model.

Journal of Alternative and Complementary Medicine 9 (2): 257-65.
14. Hayes P.Y., Lambert L.K., Lehmann R., Penman K., Kitching W. and De Voss J.J. (2007). Complete (1) H and (13) C assignments of the four major saponins from Dioscorea villosa (wild yam). Magnetic Resonance in Chemistry: MRC, 45(11), 1001-1005.
15. Higdon K., Scott A., Tucci M., Benghuzzi H., Tsao A., Puckett A., Cason Z. (2001). The use of estrogen, DHEA and diosgenin in a sustained delivery setting as a novel treatment approach for osteoporosis in the ovariectomized adult rat model. Biomedical Sciences Instrumentation 37: 281-286.
16. Hu C., Lin J., Liu S. and Yang D. (2007). A spirostanel glycoside from wild yam (Dioscorea villosa) extract and its cytostatic activity on three cancer cells. J Food and Drug Analysis 15 (3): 310- 315.
17. Ilesanmi A. O. (2009). A Handbook of Laboratory Tests for Health Practitioners. J. Ochei, A. Emeribe, T. Anifowose, A. Onyeaghala and Z. A. Jeremiah (Eds), Claverianum Centre, Bodija, Ibadan, pp110- 161.
18. Kelmanson J. E., Jager A. K. and Staden, J. van, (2002). Zulu medicinal plants with antibacterial activity. Journal of Ethnopharmacology 69(3): 241-246
19. Komesaroff P.A., Black C.V.S., Cable V. and Sudhir K. (2009). Effects of wild yam extract on menopausal symptoms, lipids and sex hormones in healthy menopausal women. Climacteria 4: 144-150.
20. Liu R.H. (2004). Potential synergy of phytochemicals in cancer prevention. Mechanism of action. J. Nutrition 134: 3478-3495.
21. Marker R. E. (1940). Sterols. CVIII. The preparation of dihydroandosterone and related compounds from diosgenin and tigogenin. J. Am.Chem. Soc. 62(10): 2621- 2625.
22. McKee Dwight, (2008). Herb, Nutrient and Drug interactions: Clinical implication and Therapeutic Strategies medical and psychosocial correlates: Results of the Massachusetts Male Aging Study. J. Urol. 151(1): 54-61.
23. Moerman D.E. (1998). Native American ethnobotany. Portland, OR: Timber Press.
24. Morgan M. (2011). Herbs for the treatment of dysfunctional uterine bleeding. A Phytotherapist's Perspective 45 (May): 1-3.
25. Oda K., Matsuda H., Murakami T., Katayama S., Ohgitani T and Yoshikawa M. (2000). Adjuvant and haemolytic activities of 47 saponins derived from medicinal and food plants. BioChem. 281: 67-74.
26. Okukpe KM., Adeloye AA., Alli OI., Belewu MA and Adeyina OA. (2012a). Physiological response of West African Dwarf Goats to graded levels of Tribulus terrestris extract. Medicinal Plants 3(4): 273- 277.
27. Okukpe KM., Adeloye AA., Abayomi FD., Alli OI., Belewu MA. and Adeyina OA. (2012b). Physiological effects of Myristica fragrans extract on West African Dwarf Bucks. Medicinal Plants 4(1): 1- 5.
28. Qureshi S., Shah AH., Tariq M. and Ageel AM. (1989). Studies on herbal aphrodisiacs used in Arab system of medicine. Am. J. Chin. Med. 17(1-2): 57- 63.
29. Raju J. and Bird R. (2007). Diosgenin, a naturally occurring furostanol saponin suppresses 3-hydroxy-3-methylglutaryl CoA reductase expression and induces apoptosis in HCT-116 human colon carcinoma cells. Cancer Letters 255(2): 194- 204.
30. Sautour M., Mitaine-Offer A.C. and Lacaille-Dubois M.A. (2007). The Dioscorea genus: a review of bioactive steroid saponins. Journal of Natural Medicines 61(2): 91-101.

31. Sautour M., Miyamoto T., Lacaille-Dubois M. A. (2006). Steroidal saponins and flavan-3-ol glycosides from Dioscorea villosa. Biochemical Systematics and Ecology 34(1): 60- 63.
32. Scott A., Higdon K., Benghuzzi H., Tucci M., Cason Z., England B., Tsao A. (2000). TCPL drug delivery system: the effects of synthetic DHEA and Diosgenin using an ovariectomized rat model. Biomedical Sciences Instrumentation 36: 171- 176.
33. Son S., Kim J.H., Sohn H.Y., Kun H.S., Kim J.S. and Kwon C.S. (2007). Anti-oxidative and Hypolipidemic Effects of Diosgenin, a Steroidal Saponin of Yam (Dioscorea spp.), on High-Cholesterol Fed Rats. Biosc. Biotechnol. Biochem. 71(12): 3063- 3071.
34. Steel R.G.D. and Torrie J.H. (1990). Principles and procedures of statistics. A biometrical approach. 2nd Ed. McGraw-Hill Books Co., New York, U.S.A. p. 633.
35. Tajuddin S., Ahmad S., Latif A., Qasmi I.A. and Amin K.M. (2005). An Experimental Study of Sexual Function Improving Effect of Myristica fragrans. Houtt. BMC Complement Altern Med. 5:16.
36. Tucci Michelle and Benghuzzi Hamed. (2003). Structural changes in the kidney associated with ovariectomy and diosgenin replacement therapy in adult female rats. Biomedical Sciences Instrumentation 39: 341- 346.
37. Watson Cynthia, (1993). Love Potions - a guide to aphrodisiacs. Optima Books. 1993. ISBN No. 0-356-21049-9.
38. Wojcikowski K., Wohlmuth H., Johnson D. nd Gobe G. (2008). Dioscorea villosa (wild yam) induces chronic kidney injury via pro-fibrotic pathways. Food and Chemical Toxicology 46(9): 3122- 3131.
39. Wren R.C. (1994). Potter's New Cyclopaedi of Botanical Drugs and Preparations, rewritten by E.M. Williamson and F.J. Evans: 1994, pub. C.W. Daniels. ISBN 0-85207-197-3.
40. Wu W.H., Liu L.Y., Chung C.J., Jou H.J. and Wang T.A. (2005): Estrogenic Effect of Yam Ingestion in Healthy Postmenopausal Women. Journal of the American College of Nutrition 24(4): 235 - 243.
41. Yen M.L., Su J.L., Chien C.L., Tseng K.W., Yang C.Y., Chen W.F., Chang C.C. (2005). Diosgenin Induces Hypoxia-Inducible Factor-1 Activation and Angiogenesis through Estrogen Receptor-Related Phosphatidylinositol 3-kinase/Akt and p38 Mitogen-Activated Protein Kinase Pathways in Osteoblasts. Molecular Pharmacology 68(4): 1061 - 1073.
42. Zava D.T., Dollbaum C.M. and Blen M. (1998). Estrogen and progestin bioactivity of foods, herbs and spices. Proc. Soc. Exp. Biol. Med., 217 (3): 369- 378.

