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GOSSYPOL CONTENT ON LEAVES AND SEEDS FROM SOME WILD *MALVACEAE* SPECIES.

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Abstract

Ten Malvaceae species were analysed for their seed and leaf gossypol content by HPLC. The results showed that gossypol is common in most of the Malvaceae family, but its concentration varies among the species and also among the varieties of the same specie. We found that *Hampea integerrina* Schldt., had two fold more gossypol in its seeds than *Gossypium hirsutum* L., from which the compound was initially isolated and its antifertility effect studied. The toxic effects of gossypol earlier observed against several parasitic protozoa and viruses makes these findings very important, since the Malvaceae specimens studied here

Afr. J. Trad. CAM (2005) **2 (1):** 4 - 12

have been used in traditional medicine against scalp infection, dysentery, gonorrhea and as antiseptic. On the other hand, it is also noteworthy that in *Hibicus sabdariffa* L., flower, traditionally used in refreshing drinks, no gossypol was detected in its seeds or leaves.

Key words: Malvaceae, gossypol, *Hibicus*, *Malvaviscus*, *Hampea*, *Anoda*, *Pavonia*, *Gossypium*, Traditional Medicine.

Introduction

Gossypol is a polyphenolic dimer sequisterpene derivative, originally extracted from *Gossypium hirsitum* L., (Malvacea) seeds. Its pharmacological characteristics have been studied mainly in relation to its reversible antifertility effects in men (National Coordinating Group on Male Antifertility Agents, 1978), without significant undesirable effects in short-term use. It has been mentioned, that other members of the *Malvaceae* family have also been shown to have antifertility effects such as *Malvaviscus conzanttii* MVC. (Joshi et al., 1981), however that activity has been attributed to the malvidin chloride, a colouring agent extracted from the flowers, no gossypol content has been reported (Bharagava, 1990). Although, *Hibicus rosa-sinensis* L., has been shown to have antifertility activity in male (Kholkute et al., 1974) and female rats (Singh et al., 1982), however its possible gossypol content has not been determined.

Gossypol has also been shown to have *in vitro* and *in vivo* inhibitory activities against diverse pathogenic agents, such as *Trypanosoma cruzi* (Montamat et al., 1982; Abe et al., 2004), *Plasmodium falciparum* (Royer et al., 1986; Tripathi et al., 2004), *Entamoeba histolytica* (González-Garza et al., 1989), *Trichomonas vaginalis* (González-Garza et al., 1995), *Giardia lamblia* (Mata-Cárdenas et al., 1998), *Taenia taemiaeformis* (Rikihisa et al., 1990), *Edwardsiella ictaluri* (Yildirim-Aksoy et al., 2004), Herpes simplex virus II (Radolf et al., 1986) and the Human Immunodeficiency Virus (Royer et al., 1991).

Gossypol is a natural racemic mixture, however the biological activities could be linked to the (-)-enantiomer (Morris et al., 1986; Tanphaichitr et al., 1988; Matlin et al.,

Afr. J. Trad. CAM (2005) **2 (1):** 4 - 12

1988; Lin et al., 1989; González-Garza et al., 1993; Joseph et al., 1986), which has been exclusively extracted in excess from *Gossypium barbadense* seeds (Jaroszewski et al., 1992; Zhou and Lin 1988).

In the present paper we report the gossypol content in nine wild species of Malvaceae used in traditional medicine for scalp infection, dysentery, gonorrhea and as antiseptic (Aguilar et al., 1994).

Material and methods

Specimens

Wild specimens were collected near Xalapa City, Veracruz, and in the state of Sinaloa México, and clasified according to Sosa and Gomez-Pompa (1994) as a floristic roll. Veracruz Flora. Instituto de Ecología A.C., Xalapa, Mex. The voucher samples where deposited in the Herbario XAL and IMSS, México. These were:

Anoda cristata L. Schltdl, Xalapa Chiltoyac, 1240 MSMN, A Hernández, E. Montoya, E, Utrera 1028, XAL. *Hampea integerrina* Schltdl,Xalapa Palenque, 1150 MSNM, Kcarroll, MG Zola B 00767, XAL.*Hibicus clypeatus*, L.Shesh Tendal, Xalapa, 1300 MSNM, Villavicencio 2, XAL *Hibicus rosa-sinensis* L.Shesh Tendal, Misantla, Ver., J Hernández, IMSS. *Hibicus sabdariffa* L.Shesh Tendal, Ahome, Sin., Grupo 3 Q:F:B: IMSS. *Malvaviscus arboreus* Cav Schltdl, Xalapa, 1300 MSNM, O Rocha, I Garcia 9, 9-B, XAL. *Malvaviscus arboreus* Cav Shltdl (Var Mex I), Xalapa, 1300 MSNM, H Villavicencio 1, XAL *Malvaviscus arboreus* Cav Schltdl (Var Mex II) Xalapa, 1140 MSNM, J Zavaleta P , A Hernandez G. 57, XAL *Pavonia schideana* Stend. J. Misantla, Ver., Hernández 96, IMSS. *Gossypium hirsutum* (L.) was supplied by ALBAMEX, S.A., México, D.F. and was used as reference.

Gossypol extraction

Leaf and seed samples were cleaned, dried at room temperature and ground. Five grams of each meal was extracted twice at room temperature for 1 h with 20 ml of 70 % aqueous

Afr. J. Trad. CAM (2005) **2 (1):** 4 - 12

acetone with continuous agitation. A third extraction does not remove any additional gossypol. The extracts were clarified by centrifugation and filtered through GVO.22µ membrane and Sepack C_{18} (Millipore Corp., Bedforf, Mass) and used for gossypol determination.

Analytical determination

The analytical determinations were made by HPLC on a Varian 5000 chromatography with a UV variable wavelength detector at 254 nm, by using a reverse-phase MCH-20 column (25 cm X 0.45 cm I. d.) from Merck, at 110 atm pressure. Acetonitrile-water (92:8) , 0.1 % phosphoric acid were used as mobile phase at flow-rate 1 ml/min. Ten microliters of extract were injected into HPLC column. Gossypol content in plants was calculated by comparison of electronically integrated areas with those obtained from gossypol standard curve (0.002 - 0.020 mg/ml). The standard curve was prepared and used within 24 h. Each analysis was made by triplicate from three different extracted samples.

Chemicals

Gossypol acetic acid used as standard was obtained from Sigma Chemical Co. (St. Louis, Mo.). All solvents were HPLC grade from E. Merck AG (Darmstadt, Germany). Filtered columns and paper from Millipore Corp. (Bedford, Mass).

Results and discussion

In eight of ten *Malvaceae* species studied, gossypol was detected in seeds (Table 1), but only in four species could it be found in the leaves as well. In one of them, *Hampea. integerrina*, showed the highest gossypol concentration in the seeds (two fold higher than *Gossypium hirsutum*), which are tall trees that grow abundantly in rainforest, whose leaves are traditionally used for wounds.

On the other hand, no gossypol was detected in either the leaves or seeds of *Hibiscus sabdariffa*. It is noteworthy that its flower (Jamaica, is the common name in México), is

Afr. J. Trad. CAM (2005) **2 (1):** 4 - 12

Table 1. Gossypol content mg/ 100 g dried sample on ten species of Malvaceas determined by HPLC.

Species	Seeds	Leaves
Anoda cristata L. Schltdl	27.24	3.52
Hampea integerrina Schltdl	1180.0	0.0
Hibicus clypeatus L.Shesh Tendal	4.37	0.0
Hibicus rosa-sinensis L.Shesh Tendal	2.05	1.87
Hibicus sabdariffa L.Shesh Tendal	0.0	0.0
Malvaviicus aboreus I Cav Schltdl	42.69	0.0
Malvavicus arborues II Cav Schltdl	0.0	0.0
Malvavicus arboreus Cav Schltdl	4.47	0.75
Pavonia schideana Stend. J.	3.33	0.0
Gossypium hirsitium L.	847.00	*297.00*

Table 2. Nine species of Malvaceas studied and its Traditional Medicinal used in México.

Anoda cristata L. Schltdl [1,2]	A= (a,b,f,): B= (h)
Hampea integerrina Schltdl [5]	(f)
Hibicus clypeatus L.Shesh Tendal	----
Hibicus rosa-sinensis L.Shesh Tendal [3]	A = (i): B = (h)
Hibicus sabdariffa L.Shesh Tendal [3]	(j)
Malvaviicus aboreus Cav Schltdl I[1,2,3]	A= (f,k,l) :B = (h,e,m,n)
Malvavicus arborues Cav Schltdl II[1,2,3]	A= (f,k,l) :B = (h,e,m,n)
Malvavicus arboreus Cav Schltdl [1,2,3]	A= (f,k,l) :B = (h,e,m,n)
Pavonia schideana Stend. J.[4,5]	A= (b,c,): B = (e,d,g)
Gossypium hirsitium L.,	--------

*Scalp infection (a), lost hair (b), dandruff (c), stomach pain (d), dysentery (e), wounds (f), haemorrhage (g) , cough (h), oral candidiasis (i), diuretic (j), antiseptic (k), gonorrhoea (l), whooping cough (m), asthma (n). [1]Stem, [2]leaves, [3]flower, [4]root, [5]whole plant. A = local administration, B = oral administration

traditionally used as refreshing drinks in México and several countries. This result complements the report of Jaroszewski et al., (1992) where no gossypol was found in the aerial parts of *H sabdariffa*.. The other two species studied of the same genus, *H. clypeatus* and *H. rosasinesis* had detectable amounts of gossypol in their seeds and leaves respectively. This, and the variability of the gossypol content found in the varieties of *Malvaviscus arboreus,* show a great diversity of gossypol presence or quantity of in the Malvaceae family, and that this compound is not exclusive of the genera *Gossypium.*

Other Malvaceae, like *Montesuma speciosissima* (Joland et al., 1975), *Thepesia populnea* (Cass et al., 1991), *Azanza lampa, A garckeana,* and *Cienfuegosia drummondia* have been shown also to contain gossypol, it is interesting to mention that in all of them there has been reported an excess of (+) gossypol (Jaroszewski et al., 1992).

Several species of Malvaceae family have been use in Mexican Traditional Medicine (Aguilar et al., 1994), for several infectious diseases (Table 2), however their active principle(s) has not been elucidated. It is possible that compounds other than gossypol could be responsible for the antiparasitic activity.

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References

1. Abe F, Nagafuji S, Okabe H, Akahane H, Estrada-Muniz E, Huerta-Reyes M, Reyes-Chilpa R.2004. Trypanocidal constituents in plants 3. Leaves of *Garcinia intermedia* and heartwood of *Calophyllum brasiliense* Biol Pharm Bull. **27:**141-3.

2. Aguilar, A., Camacho, J. R., Chino, S., Jacquez, P. and López, M.E. (1994).Herbario Medicinal del Instituto Mexicano del Seguro Social (Instituto Mexicano del Seguro Social, ed.) México.
3. Bharagava, S.K. (1990). Anti-spermatogenic activity of malvidin chloride in langur monkeys (Presbytis entellus entellus Dufresne). Int J Androl **13:** 207-215.
4. Cass, Q. B., Tiritan, E., Matlin, S.A. and Freire, E.C. (1991). Gossypol enantiomer ratios in cotton seeds. Phytochemistry **30:** 2655-2657.
5. González-Garza, M. T., Mata-Cárdenas, B. D. and Said-Fernández, S. (1989). High susceptibility of five axenic *Entamoeba histolytica* strains to gossypol. Trans R Soc Trop Med Hyg **83:** 522-524.
6. González-Garza, M.T., Matlin, S.A., Mata-Cárdenas, B.D. and Said-Fernández, S. (1993). Differential effects of the (+)- and (-)-gossypol enantiomers upon *Entamoeba histolytica* axenic cultures. J Pharm Pharmacol **45:** 144-145.
7. González-Garza, M.T., Castro-Garza, J. and Said-Fernández, S. (1995) Growth inhibition of *Trichomonas vaginalis* by gossypol. Pharm Sci **1:** 39-40.
8. Jaroszewski, J.W., Strom-Hansen, T., Hansen, S.H., Thrastrup, O. and Kofod, H. (1992) On the botanical distribution of chiral forms of gossypol. Planta Med. **58:** 454-458.
9. Joland, D., Weidhopf, R. M. and Cole, J. R. (1975). Tumor inhibitory agent from *Montesuma speciocissima* (Malvaceae). J Pharmaceu Sci **64:** 1889-1890.
10. Joseph, A. E. A., Matlin, S. A. and Knox, P. (1986) Cytotoxicity of enantiomers of gossypol. Bri J Cancer **5:** 511-513.
11. Joshi, B. C., Sabtish, K., Verma, O. P., Chatterjee, S. N. and Jacob, D. (1981). Antifertility effects of chronically administered *Malvaviscus conzatii* flower extract on male albino mice. Planta Med. **41:** 274-280.
12. Kholkute, S. D., Chartterjee, S., Srivastva, D. N. and Udupa, K. N. (1974). Proceedings: Effect of *Hibicus rosa-sinensis* on the reproductive organs of male rats. J Reprod Fert **38:** 233-234.

13. Lin, T.S., Schinazi, R., Griffith, B. P., August, E. M., Eriksson, B. F. H., Zheng, D. K., Huang, L. and Prusoff, W. H. (1989). Selective inhibition of human immunodeficiency virus type 1 replication by the (-) but not the (+) enantiomer of gossypol. Antimicrob Agents Chemother **33:** 2149-2151.

14. Mata-Cardenás, B.D., Vargas-Villarreal, J., Martinez-Rodríguez, Navarro-Marmolejo L, González-Garza, M.T. and Said-Fernández, S. (1998). *In vitro Giardia lamblia* growth inhibition by gossypol. Pharm Pharmacol Comm **4:**361-363.

15. Matlin, S.A., Zhou, R.H. and Belenguer, A. (1988). Large-scale resolution of gossypol enantiomers for biological evaluation. Contraception **37:** 229-237.

16. Montamat, E.E., Burgos, C., Gerez de Burgos, N.M., Rovai, L.E. and Blanco, A. (1982) Inhibitory action of gossypol on enzymes and growth of *Trypanosoma cruzi*. Science **218:** 288-289.

17. Morris, I.D., Higgins, C. and Matlin, S. A. (1986). Inhibition of testicular LDH-X from laboratory animals and man by gossypol and its isomers. Journal of Reproduction and Fertility **77:** 607-612.

18. National Coordinating Group on Male Antifertility Agents. (1978). Gossypol a new antifertility agent for males. Chin Med. J. **4:** 417-428.

19. Radolf, R., Deck, L. M., Royer, R. E. and Vander Jagt, D. L. (1986). Antiviral activities of gossypol and its derivatives against herpes simplex virus type II. Pharmacol Res. Comm .**18:** 1063-1073.

20. Rikihisa, Y., Lin, Y.C., Garber, P.L. and Gu, Y. (1990). *Taenia taeniaeformis*: Inactivation of metacestodes by Gossypol in vitro. Exp Parasitol **71:** 135-145.

21. Royer, R.E., Deck, L.M., Campos, N.M., Hunsaker, L.A. and Vander Jagt, D.L. (1986) Biologically active derivates of gossypol: Synthesis and antimalarial activities of peri- acylated gossylic nitriles. J. Med. Chem. **29:**1799-1801.

22. Royer, R. E., Mills, R. G., Deck, L. M., Mertz, G. J. and Vander Jagt, D. L. (1991). Inhibition of Human Immunodeficiency virus type I replication by derivatives of gossypol. Pharmacol Res **24:** 407-412.

23. Singh, M. P., Singh, R.H. and Udupa, K. N. (1982). Antifertylity activity of benzene extract of *Hibicus rosa-sinensis* flowers on female albino rats. Planta Med. **44:** 171-174.

24. Sosa, V. and Gómes-Pompa, A. (1994). Floristic Roll. . Flora de Veracruz. Instituto de Ecología, A.C. Ed, México.

25. Tanphaichitr, N., Fitzgerald, L. M.. and Matlin, S. A. (1988). Differential effects of (+) and (-) enantiomers on mitochondrial functions proliferation of cultured TM_4 cells. J Androl. **9:** 270-277.

26. Tripathi A. K., Desai, P. V., Pradhan, A., Khan, S. I., Avery, M. A. Walker, L. A. and Tekwani, B. L. (2004). An alpha-proteobacterial type malate dehydrogenase may complement LDH function in *Plasmodium falciparum.* Eur. J. Biochem. **271:**3488-502.

27. Yildirim-Aksoy, M., Lim, C., Dowd, M. K., Wan, P. J. Klesius, P. H. and Shoemaker, C. (2004). *In vitro* inhibitory effect of gossypol from gossypol-acetic acid, and (+)- and (-)-isomers of gossypol on the growth of *Edwardsiella ictaluri*. J Appl Microbiol. **97:**87-92.

28. Zhou, R. H. and Lin, X. D. (1988). Isolation of (-)-gossypol from natural plant. Contraception 37: 239-245.