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
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 hirsutum* 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 taeniaeformis* (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.,
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 classified 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.
- Hibiscus clypeatus, L. Shesh Tendal, Xalapa, 1300 MSNM, Villavicencio 2, XAL.
- Hibiscus rosa-sinensis L. Shesh Tendal, Misantla, Ver., J Hernández, IMSS.
- Hibiscus 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 Schltdl (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
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., Bedford, 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
Table 1. Gossypol content mg/100 g dried sample on ten species of Malvaceas determined by HPLC:

<table>
<thead>
<tr>
<th>Species</th>
<th>Seeds</th>
<th>Leaves</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anoda cristata L. Schltdl</td>
<td>27.24</td>
<td>3.52</td>
</tr>
<tr>
<td>Hampea integerrina Schldtl</td>
<td>1180.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Hibicus clypeatus L. Shesh Tendal</td>
<td>4.37</td>
<td>0.0</td>
</tr>
<tr>
<td>Hibicus rosa-sinensis L. Shesh Tendal</td>
<td>2.05</td>
<td>1.87</td>
</tr>
<tr>
<td>Hibicus sabdariffa L. Shesh Tendal</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Malvavicus aboreus ICav Schldtl</td>
<td>42.69</td>
<td>0.0</td>
</tr>
<tr>
<td>Malvavicus arborues II Cav Schldtl</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Malvavicus arboreus Cav Schldtl</td>
<td>4.47</td>
<td>0.75</td>
</tr>
<tr>
<td>Pavonia schideana Stend. J.</td>
<td>3.33</td>
<td>0.0</td>
</tr>
<tr>
<td>Gossypium hirsitium L.</td>
<td>847.00</td>
<td>297.00</td>
</tr>
</tbody>
</table>

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

Anoda cristata L. Schltdl ¹² 
A = (a,b,f,): B = (h)

Hampea integerrina Schldtl ⁵
(f)

Hibicus clypeatus L. Shesh Tendal
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Hibicus rosa-sinensis L. Shesh Tendal ³
A = (i): B = (h)

Hibicus sabdariffa L. Shesh Tendal ³
(j)

Malvavicus aboreus Cav Schldtl ¹²,³
A = (f,k,l) : B = (h,e,m,n)

Malvavicus arborues Cav Schldtl ¹²,³
A = (f,k,l) : B = (h,e,m,n)

Malvavicus arboreus Cav Schldtl ¹²,³
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.,
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*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). ¹Stem, ²leaves, ³flower, ⁴root, ⁵whole 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 *Malaviscus 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 used 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.

**Acknowledgments**

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**References**


