

Biological Activity of Withanolides

R D Budhiraja*, Pawan Krishan and S Sudhir**

Department of Pharmacy and Pharmacology**, Pt B D Sharma PGIMS, Rohtak 124 001

Received: 24 March 2000; accepted: 18 July 2000

A large number of withanolides are isolated from solanaceous plants and some derivatives are also prepared. Some of the withanolides, mainly Withaferin A(WA), Withanolide D(WD), Withanolide E(WE), 3 β -hydroxy-2,3-dihydrowithanolide F, and sitoindosides are assessed for biological activities. These compounds and some other withanolides are found to have useful biological effects such as anticancer, radiosensitizing, antibacterial, adaptogenic, immunomodulating, antioxidant, hepatoprotective, antistress, antiinflammatory antiarthritic, and insect antifeedant activities. However, none of the withanolide has so far been evaluated for pharmacokinetic and pharmacodynamic properties. The biological activities of the withanolides vary with the substitution pattern of the compounds. Further, several withanolides are present in each plant. Thus the detailed pharmacological studies of the withanolides are warranted before any of these compound can be clinically assessed and used.

Introduction

Withanolides are a group of C28 steroidal lactones isolated from various solanaceous plants. First compound of this group was isolated first by Kurup from the leaves of an Indian plant called *Withania somnifera* (Ashwagandha) and later on isolated from the Israeli variety of the plant, characterised and given the name Withaferin A (WA) (ref. 3) (Figure 1). Some semi-synthetic derivatives have also been prepared by interconversions in the withanolide series.

Some of the withanolides and their derivatives are found to possess very interesting biological activities *in vitro* and *in vivo*, especially the antimicrobial, antitumour, radiosensitizing, anti-inflammatory, adaptogenic, immunomodulating, antioxidant, antistress and insect antifeedant. So far the most important withanolide reported to show these effects without noticeable systemic toxicity is WA and has been contemplated for use in cancer therapy and radiosensitizing agent. In view of the useful biological activities, intensive research is underway on the plants containing withanolides to isolate new and more potent drugs of this series.

Since the last review of biological activity of the withanolides⁴, some important reports have appeared about these activities. These studies are very interesting and the present paper attempts to update the biological activity of withanolides. Chemical structure and biological activity relationship have also been discussed, based on the available information.

Antimicrobial Activity

Antimicrobial activity of first steroidal lactone identified as WA was reported by Kurup^{1,2} which has been confirmed by others⁵⁻⁷. Some other withanolides have also been reported to have antibacterial activity

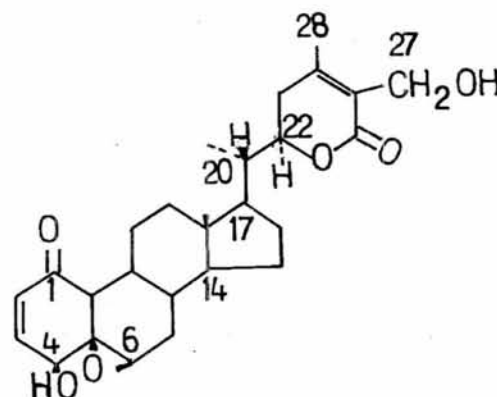


Figure 1 — Withaferin A

* Corresponding author

and these are active against gram-positive bacteria but not against gram-negative and anaerobic bacteria or non-filamentous fungi^{4,6-10}. WA (ref. 11) and a new withanolide isolated from *W. coagulans* and identified as 17 β -hydroxywithanolide K (2S, 22R)-14 α , 17 β , 20 β -trihydroxy-1-oxo-witha-2,5,24-trienolide have been found to be active against several potentially pathogenic fungi¹².

The mechanism of action of antibacterial activity of WA appears to be due to its reaction with -SH group of the enzymes or other metabolites essential to the organisms. The antibacterial activity of WA was inhibited by glutathione and cysteine due to reaction with carbonyl group of the lactone.

Based on the results of the antibacterial activity reported^{6,13,14}, it can be concluded that unsaturated lactone and 5,6-epoxide system appear to be essential for antibacterial activity which is based on the following facts:

(i) Hydrolysis and hydrogenation of the lactone caused loss of antibacterial activity. Acetylation of 27-OH did not affect the activity. Further, deoxy WA and withanolide (WD) which do not contain 27-OH are active^{13,14}.

(ii) 5,6 epoxide system seems to be essential as the lactones like Withanone, Nicandrenone, physalin A and B having no 5,6 epoxide are not active but WA, WD and withanolide (WE) having 5,6 epoxide system are active against bacteria. Lactones with 6,7-epoxy group (withanone and nicandrenone) are not active.

(iii) Hydrogenation of double bond at C₂-C₃ reduced the antibacterial activity as dihydrowithaferin A is less active than WA.

Antitumour Activity

WA and some other withanolides have been found to possess antimitotic and anticancer effects, both *in vitro* and *in vivo* studies. Kupchan *et al.*¹⁵ were the first to report the growth inhibitory effect of WA *in vitro* against cells from human carcinoma of the nasopharynx (KB) and on Sarcoma-180 (S-180) in mice.

Shohat *et al.*¹⁶⁻¹⁸ have found that WA produced *in vitro* and *in vivo* growth retardation of Ehrlich ascites carcinoma (EAC), S-180, Sarcoma Black and E-0771 mammary adenocarcinoma. Growth of EAC was completely inhibited in more than 50 per cent of

mice which survived for more than 100 d without evidence of tumour growth. Antitumour activity was much more effective in combination with low dose of X-ray therapy. Further studies^{17,19} showed that WA rendered the cured animals refractory to reinoculation with the same tumour. The tumour regression during WA treatment was found to be due to immune stimulation characterised by the presence of circulating antibodies with toxic properties towards the subsequent tumour cells implantation. Arrest of division of carcinoma cells at metaphase with WA has been reported in EAC (ref. 17), human larynx carcinoma cells²⁰, HeLA (ref. 21) and *Allium cepa*²²;

Chakraborti *et al.*²³ have found that WD has significant antitumour activity against carcinoma of the nasopharynx *in vitro* and *in vivo* against sarcoma-180 in mice. Das *et al.*²⁴ have also found that WD has anticancer effects against EAC and Sarcoma-180 cells.

Fuska *et al.*²⁵⁻²⁷ have observed that WA and some of its derivatives suppressed *in vitro* proliferation of P-388 murine lymphocytic leukemic cells and also affected protein and nucleic acids synthesis by inhibiting the incorporation of the precursors, thymidine, uridine, and valine. The cytotoxicity was related to the structure. The double bond at position C₂-C₃ was important and dissociation of this bond decreased the cytotoxicity but dissociation of this bond at C₂₄-C₂₅ did not inhibit the activity. Pelletier *et al.*²⁸ and Keinan *et al.*²⁹ have tested some withanolide derivatives and found them active against murine p-388 lymphocytes leukemia. Gonzales *et al.*³⁰ found that seven withanolides from *W. frutescens* and *W. aristata* were active against human carcinoma HeLA-229 cells. Jaborosalactone-L and 3 α -oxygenated withanolides from *D. Penninervium* also exhibited cytotoxicity to both human and murine carcinoma³¹. A study by Haseena Begum and Sadique³² has demonstrated that WA interfered with the synthesis of sulphated mucopolysaccharides and impaired oxidative phosphorylation by enhancing ATPase activity, followed by inhibition of succinate dehydrogenase activity. The unsaturated lactone in the side chain is important for the antitumour activity of WA; 1-keto-2-ene, and the epoxide functions being essential to elicit the antitumour activity³³. Physalin A,B,D,F (ref. 34) and WE (ref. 35) also have been found to have cytotoxic effects on cancer cells.

Uma Devi^{36,37} has reported that tumour inhibitory effect of WA on EAC *in vivo* increased

with the dose at 10-60mg/kg. The dose survival curve showed a linear quadratic pattern with a sharp increase in tumour free survival from 10 to 40mg/kg. above which the slope of the curve decreased. The ED₅₀ was 33mg/kg. Repeated administration of WA showed significant growth inhibitory and cytotoxic effects on EAC. A total dose of 40 mg/kg. in 8 fraction (5 × 8mg/kg.) or a total dose of 60mg/kg. in 8 fraction (7.5 × 8mg/kg.) starting from 24 h. after tumour inoculation produced 20per cent tumour cure and tumour free survival on 120 d. When these doses were given in two fractions of 20mg/kg or 30mg/kg (total of 40 or 60mg/kg) it resulted in 70 per cent and 80per cent tumour free survivors respectively for 120 d. Multiple higher dose (40mg/kg) was not tolerated. Combination with radiation enhanced the tumour cure and tumour free survival at 120 d. WA was more effective against early tumours but combination with radiation improved the response even in advanced cases. Thus the WA + Radiation has synergistic effect with all drug schedules.

Radiosensitizing effect and cytotoxicity of WA on Chinese hamster V79 cells *in vitro* has been reported by Uma Devi *et al.*³⁸. WA has growth inhibitory effect *in vitro* on both Chinese hamster V79 cells and HeLA cells. It reduced the survival of V79 cells in a dose dependent manner. The LD₅₀ was found to be 16 μm. Further, WA treatment for 1 h before irradiation enhanced cell killing *in vitro*³⁹. A nontoxic dose of 2.1 μM gave a sensitizer ratio (SER) of 1.5 for 37 per cent survival in V79 cells. SER increased when the dose was increased to 5.25 and 10.5 μM. But at the higher dose the drug was toxic. Cell cycle studies indicated that WA induces an immediate block of cells in G2 M phase³⁹. This may be due to an effect on the mitosis rather than on G2 phase as reported by earlier workers^{17,20,21} that the drug arrested cell division in metaphase.

Sharada *et al.*⁴⁰ and Ganasoundari *et al.*⁴¹ have found that WA has growth inhibitory and radiosensitizing effects *in vivo* at 30mg/kg dose. WA increased the radiation induced bone marrow cell lethality to the same extent as cyclophosphamide. Radiosensitizing effect of WA is not tumour specific.

Structure Activity Relationship

Cytotoxicity of the withanolides is related to their structure. Shohat *et al.*¹⁶ have reported that the presence of unsaturated lactone in the chain to which

an allylic primary alcohol is attached at C-26 and the other end of the molecule may be specific chemical system possessing carcinostatic properties.

Fuska *et al.*²⁵ after testing WA and its nine derivatives found that cytotoxicity was due to double bond at position C₂-C₃ dissociation of this bond markedly decreased the cytotoxicity in all the derivatives. Dissociation of double bond at C₂₄-C₂₅ or removal of 27-OH acid did not cause any significant change in biological effects of the derivatives. An addition of carbonyl group at C₄ increased the activity of WA. It has also been reported³³ that unsaturated lactone in the side chain is important for the antitumour activity of WA; 1-keto-2-ene and the epoxide function being essential to elicit the antitumour activity.

Mechanism of Antitumour Action

The exact mechanism of antitumour and radiosensitizing properties of withanolides is not well understood. Palyi *et al.*²¹ observed that WA induced mitotic arrest in HeLA cells *in vitro*. Similar effect was also reported by Shohat *et al.*^{17,20} in EAC tumour cells and *Allium cepa* cells²².

Inhibition of RNA synthesis by WA and WD in mice in sarcoma-180 ascites tumour cells was reported by Choudhary and Neogy⁴². Later Fuska *et al.*²⁵ showed that WA inhibited DNA and protein synthesis in P-388 cells *in vitro*. This may explain the potent cytotoxic property of WA.

Shohat and Joshua¹⁹ have suggested stimulation of the immune system to explain the growth inhibitory effect on EAC. The immune stimulation could contribute to some of the antitumour effects observed *in vivo*, but the cytotoxic effects *in vitro* cannot be explained by this mechanism. Moreover, an immunosuppressive effect has been reported against adjuvant arthritis in rats, in chicken host vs graft reaction⁴³ and in human B and T lymphocytes and mice thymocytes⁴⁴.

Recently, using the flow cytometry, it has been demonstrated that WA induced an accumulation of V79 cells in the G 2-M phase, most likely due to a mitotic arrest rather than an effect on G-2 phase³⁹. Radiosensitizing effect of WA observed both *in vivo*^{37,40} and *in vitro*³⁹ cannot be explained wholly on the basis of effects on cell cycle or macromolecular synthesis.

Cell cycle studies indicate that WA might interfere with DNA synthesis, leading to inhibition of repair of radiation injury, as there was a reduction in the shoulder of the V-79 cell survival curve. The drug treatment also produced an immediate though very transient increase in S-phase cells³⁹.

Immunomodulating Effects

Withanolides have been reported to possess both immunostimulating and immunosuppressive effects in different studies. WA has been found¹⁹ to inhibit the growth of EAC in mice within 24 h followed by complete disappearance of tumour cells after 3-4 d of treatment by activating the immune response as it caused a striking proliferation of macrophages in the peritoneal cavity and clustering of acid phosphatase rich macrophages around EAC. WA cured mice exhibited complete tumour regression after reinoculation of EAC. The observations that WA acts by immune activation was confirmed when in normal mice the growth of EAC was prevented by passive transfer of serum or peritoneal cells but not of spleen cells taken from immune mice. Rejection of EAC by the sucklings born to immune mothers could be due to passive transfer of circulating antibodies through the milk. WA caused activation of humoral antibodies was further demonstrated by complement fixation, passive cutaneous anaphylaxis and cytotoxic tests.

Immunosuppressive activity of WA was observed when tested against adjuvant arthritis in rats and it inhibited graft versus host reaction in chickens⁴³.

WA and WE (ref. 44) showed immunosuppressive effects on human B and T lymphocytes and mice thymocytes. Both these compounds prevented the formation of *E. rosettes* and EAC rosettes by normal human B and T lymphocytes. WA alone could inhibit the formation of red blood rosettes by chronic lymphatic leukemic cells. WE had a specific effect on T lymphocytes whereas WA affects both T and B lymphocytes. Both the lactones were found to be effective in suppressing local xenogenic graft versus host reaction.

Bahr and Hansel⁴⁵ have tested 5,20 α -(R)-dihydroxy-6 α ,7 α -epoxy-1-oxo (5 α) with 2, 24-dienolide for its immunomodulating effects in spleen cell cultures and have found that the compound has immunosuppressive effect. Immunosuppressive acti-

vities of jaborosalactone and three 16 α -oxygenated withanolides from the leaves of *D. penninervium* have been studied by Habtemariam³¹.

Anbalagan and Sadique⁴⁶ have found that inflammation induced with various agents reflected in the production of modulator protein by liver. Withanolides acted as regulator for modulator protein synthesis. WD and WE have been found to have antineoplastic and immunosuppressive effect³⁵. Sitoindosides IX and X (Glycowithanolides) from *W. somnifera*⁴⁷ and acnistin A, B and E from *Dunalia solanaceae*⁴⁸ are reported to have immunomodulatory effect.

Antiinflammatory and Antiarthritic Activity

WA has been found to have antiinflammatory and antiarthritic activity⁴⁹. Sethi and Subramanian⁵⁰ have assessed WA, withanone, withanolide (mp 283-85 °C) and a new withanolide (mp 212 °C) by acute and subacute models of antiinflammatory effects. It was found that there is significant variation in the biological activity of the withanolides. WE and related compounds⁵¹ and 3 β -hydroxy-2,3-dihydro-withanolide F (ref. 52) have been demonstrated to have antiinflammatory effects against acute and subacute models of inflammation. 24,25-epoxy-vitanolide D from *Physalis angulata* has been reported to have antiinflammatory effect⁵³.

It has been found that withanolide fraction of the aerial parts of Iraqi *W. somnifera* at 10 mg/kg dose i p had antigranuloma activity. The fraction reduced the weight of adrenal glands but not of thymus or spleen. The exact mechanism of action was not established but the effects were attributed to WA (ref. 54). WA has also been shown to have granuloma and adrenal inhibiting activity without affecting spleen and body weight⁵⁵.

Hepatoprotective Effect

WA (ref. 56) at 10 mg/kg i p dose has been found to possess significant protective effect in rats against CCl₄-induced hepatotoxicity, when assessed by observing the pentobarbitone, (30 mg/kg) induced hypnosis, levels of SGOT, SGPT, alkaline phosphatase, serum proteins and histopathological examination of the hepatic tissue. In the withanolides treated rats the liver cells looked almost normal and there was marked reduced fatty infiltration and

sinusoidal dilatation as compared to control. The withanolides were equally or slightly more active than hydrocortisone on weight basis. 3β -hydroxy-2,3-dihydrowithanolide F also has hepatoprotective effect⁵⁷. Total withanolides from *W. somnifera* showed hepatoprotective effect, decreased the levels of peroxidation products and increased the levels of antioxidant enzymes. Mechanism of protective effect is yet not known but may be due to antioxidant effects of the withanolides⁵⁸.

Antioxidant Activity

Equimolar mixtures of sitoindosides VII-X and WA from *W. somnifera* were tested in rat brain for superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) and results compared with deprenyl, an antioxidant. Active glycowithanolides at 10 and 20 mg / kg i p dose for 21 d caused dose related increase in SOD, CAT and GPX activity, and results were comparable with 2 mg / kg dose of deprenyl ip. It has been claimed that antioxidant effects of these compounds may partly explain the antistress, immunomodulatory, cognition-facilitating, antiinflammatory and anti-aging effects of *W. somnifera*⁵⁹. Withaperuvine E, also has antioxidant activity⁶⁰. Withanolides fraction of *W. somnifera* has been found to have effects on peroxidation products and antioxidant enzymes⁵⁸.

Antifeedant Effect

Ascher *et al.*⁶¹ have assessed the insect antifeedant effects of many withanolides and related steroids from solanaceae. It was found that WE as well as related steroids have antifeedant properties against larvae of *Spodoptera littoralis*. Nic-1 (nicandrenone)⁶² was found to be a feeding deterrent for larvae of *Manduca sexta* and *Pectinophora gossypiella* but not effective against *S. littoralis*. WE was found to be most potent antifeedant whereas WA, WD and Physalin B were found to be inactive at 0.01 per cent concentration regarding antifeedant properties.

Eight steroidal lactones were tested by Ascher *et al.*⁶³ for their antifeedant efficacy for larvae of *Epilachna varivestis* and found that Nicalbin A (NA), WE, 4β -hydroxy WE, Nicandrenone, Nicalbin B and some other steroids were found to be active against

E. varivestis. Antifeedant properties on *S. littoralis* larvae of leaves of *W. somnifera* of chemotype III were found to be due to the presence of WE. NA was potent antifeedant. Antifeedant effects of *W. somnifera* against *S. littoralis* was further found to be due to WE and WD (ref. 64).

The antifeedant effects of several new withanolides against larvae of *S. littoralis*, *E. varivestis* and *T. Castenium* were investigated by Ascher *et al.*⁶⁵. It was demonstrated that 2,3-dihydroWE have antifeedant effect for *S. littoralis* and *E. varivestis*, NA and some other steroids were found to be active against *E. varivestis* and *T. castenium*.

Nic-1 and Nic-2 (Nicandrenone) having the antifeedant effect are the steroidal lactones isolated from *Nicandra physaloides*⁶⁶. Keinan *et al.*²⁹ found that some derivatives of WA also have antifeedant properties.

Comparison of the antifeedant results with different compounds showed that the substitution pattern of Carbon 4, 5 and 6 is critical for antifeedant activity. Addition of a β -oriented hydroxyl group at position 4 reduced the activity by a factor of ten whereas hydrolysis of the 5,6-epoxide to 5,6-diol system in the withanolide led to nearly total inactivation⁶¹.

Cardiovascular System

The withanolides have a close structural similarity with aglycones of the cardiac glycosides in possessing a 6-membered unsaturated lactone ring attached to a steroidal ring at C-20 instead of at C-17. The 3β -hydroxy 2,3-dihydro withanolide F has been reported to produce moderate fall in dog BP which was blocked with atropine^{67,68}.

Haemopoietic System and Bone Marrow

Withanolides have been reported to have effect on haemopoietic system and bone marrow. WA at 30 mg/kg reduced the stem cell of bone marrow but found to be less toxic than the cytotoxic drug cyclophosphamide (CP). WA increased the radiation induced bone marrow lethality to the same extent as CP suggesting that the ratio sensitization effect of WA is not tumour specific⁴¹.

Total withanolides have been reported to increase haemoglobin and RBC count but no significant effect on bone marrow⁶⁹, SGOT, SGPT, and alkaline phosphatase⁵⁸.

Effect on CNS

Glycowithanolides have been found to have effect on CNS. It has been reported that equimolar mixture of sitoindosides VII-X and WA from *W. somnifera* at 40 mg/kg. ip when injected in rats for 7 d led to differential effects on acetylcholinesterase (AChE) activity in the basal forebrain nuclei, slightly enhanced AChE activity was found in the lateral septum and globus pallidus, whereas in the vertical diagonal band AChE activity was reduced. Number of M₁ and M₂ receptors also increased in some areas of the brain but did not affect GABA and benzodiazepine receptors. The drug induced increase in cortical muscarinic acetylcholine receptors may partly explain the memory improving effects of *W. somnifera* observed in animals and humans⁷⁰. 3- β -hydroxy 2,3-dihydro withanolide F at dose of 5-10 mg/kg i p did not show any change in general behaviour, hypnotic activity, and analgesic or hypothermic activity⁵².

Bhattacharya *et al.*⁷¹ have found that the WA and sitoindosides VII-X, isolated from *W. somnifera* reversed the ibotenic acid induced cognitive defects in Alzheimers disease model. Ghosal *et al.*^{72,73} have also found that sitoindosides IX-X exhibited adaptogenic and immunostimulatory activity. Sitoindosides VII and VIII have antistress effects⁷⁴ in all the test parameters used. Glycowithanolides have shown effects on morphine induced inhibition of intestinal motility and tolerance to analgesia in man⁷⁵.

Adverse Effects

So far withanolides have not been assessed for adverse effects. However, few reports are available about the acute toxicity of some withanolides. Sharada *et al.*^{40,76} reported that the approximate LD₅₀ of WA in swiss mice is 80 mg/kg. Dose above 100 mg/kg produced severe toxic symptoms like ruffling of hair, diarrhoea, and severe weight loss and no animals survived after injection of 150 mg/kg.

However, LD₅₀ for WA reported by other workers are as 54 mg/kg, 120 mg/kg and even 400 mg/kg ip (Shohat *et al.*¹⁶). For some other withanolides LD₅₀ reported is: peruviana 60 mg/kg., withanone more than 400 mg/kg. Acute toxicity of glycowithanolides (Sitoindosides) indicate that these have low order toxicity. LD₅₀ of sitoindosides X and X by ip route was 518 \pm 34 mg/kg and 808 \pm 60 mg/kg respectively⁷⁴. LD₅₀ of sitoindosides VII and VIII is about 1564 and 92 mg/kg⁴⁷, respectively.

Conclusions

The present study reveals that the withanolides are a new group of steroidal lactones. Many of these possess interesting biological activities such as antitumour, radiosensitizing, antibacterial, antiinflammatory, antioxidant, immunomodulatory and insect antifeedant. Some of the compounds may prove useful for any of the above conditions. WA has already been contemplated for clinical trials as anticancer and radiosensitizer. But so far none of the withanolide has been marketed for medicinal or any other use.

Pharmaceutical industry is using *W. somnifera* for the manufacture of many Ayurvedic preparations being used as tonic and health food over long periods in all age groups both by men and women including during pregnancy and lactation. Contrary to this, WA, WD and WE from this plant have been found to have anticancer and radiosensitizing effects. Anticancer drugs are well documented to produce harmful effects during pregnancy and lactation. Therefore, these studies are a challenge to the future scientists and drug control administration to find out how safe is *W. somnifera* found to be anticancer; and the preparations containing *W. somnifera* to be used as tonic and health food during pregnancy and lactation and at the same time as anticancer drug.

These studies may also prove useful in Pharmaceutical industry to help in quality control of the medicines containing this plant or other plants containing withanolides to ensure that the active constituents are present in sufficient amounts to produce appropriate effects. Withanolides may also prove useful as raw material for the synthesis of other steroidal drugs.

References

- 1 Kurup P A, *Curr Sci*, **25** (1956) 57.
- 2 Kurup P A, *Antibiot Chemother*, **8** (1958) 511
- 3 Lavie D, Glotter E & Shvo Y, *J Chem Soc (C)*, **18** (1965) 7517.
- 4 Budhiraja R D & Sudhir S, *J Sci Ind Res*, **46** (1987) 488.
- 5 Kohlmuenzer S & Krupinska J, *Dissertationes Pharam*, **14** (1963) 501; *Chem Abstr*, **59** (1963) 6866g.
- 6 Sethi P D, Ravinderan P C, Sharma K B & Subramnian S S, *Indian J Pharm*, **36** (1974) 122.
- 7 Ben-Efraim, S & Yarden A, *Antibiot Chemother*, **12** (1962) 576.
- 8 Das J M & Kurup P A, *Naturwissenschaften*, **50** (1963) 603.
- 9 Chatterjee S, Chakraborti S K & Antonie V, *Chem Abstr*, **83** (1980) 38210b.
- 10 Khan F Z, Saeed M A, Alam M, Chaudhary A R & Ismail M, *J Fac Pharm Gazi*, **10** (1993) 105.
- 11 Dasgupta L R, Sethi P D, Sharma K B & Subramanian S S, *Indian J Pharm*, **32** (1970) 70.
- 12 Chaothary M I, Dur-e-Shahwar, Parveen Z, Jabbar A, Ali I & Atta-ur-Rahman, *Phytochemistry*, **40** (1995) 1243.
- 13 Kurup P A, *J Sci Ind Res*, **15** (1956) 153.
- 14 Kurup P A, *Naturwissenschaften*, **49** (1962) 469.
- 15 Kupchan S M, Doskotch R W, Bollinger P, Mcphail A T, Sim G A & Renauld J A S, *J Am Chem Soc*, **87** (1965) 5805.
- 16 Shohat B, Gitter S, Abraham A & Lavie D, *Cancer Chemother Rep*, **51** (1967) 271.
- 17 Shohat B, Gitter S & Lavie D, *Int J Cancer*, **5** (1970) 244.
- 18 Shohat B Shaltiel A, Ben-Bassat M & Joshua H, *Cancer Lett.*, **2** (1976) 71.
- 19 Shohat B & Joshua H, *Int J Cancer*, **8** (1971) 487.
- 20 Shohat B, *Z Krebsforsch Klin Oncol*, **80** (1973) 97, *Chem Abstr*, **79** (1973) 139h.
- 21 Palyi I, Tyihak E & Palyi V, *Herba Hung*, **8** (1969) 73.
- 22 Shohat B & Joshua H, *Eur J Cancer*, **7** (1971) 561.
- 23 Chakraborti S K, De B K & Bandyopadhyay T, *Experientia*, **30** (1974) 852.
- 24 Das H, Datta S K, Bhattacharya B & Chakraborti S K, *Indian J Cancer Chemother*, **7** (1985) 59.
- 25 Fuska J, Fuskova A, Rosazza J P & Nicholas A W, *Neoplasma*, **31** (1984) 31.
- 26 Fuska J, Prousek J, Rosazza J & Budesinky N, *Steroids*, **40** (1982) 157.
- 27 Fuska J, Proška B, Williamson J & Rosazza A, *Folia Microbiol*, **32** (1987) 112.
- 28 Pelletier S W, Gebeyehu G, Modi N V, *Heterocycles*, **19** (1982) 235.
- 29 Keinan E, Sahai M & Kirson I, *J Org Chem*, **48** (1983) 2550.
- 30 Gonzales A G, Darias V, Herrera D A M & Suarez M C, *Fitoterapia*, **53** (1982) 85.
- 31 Habtemariam S, *Planta Med*, **63** (1997) 15.
- 32 Haseena Bagum V & Sadique J, *Biochem Med Metabol Biol*, **38** (1987) 272.
- 33 Yoshida M, Hoshi A, Kuretani K, Ishiguro M & Ikekawa N, *J Pharm Dyn*, **2** (1979) 92.
- 34 Singh S & Kumar S, *Withania Somnifera*, (Central Institute of Medicinal & Aromatic Plants, Lucknow) 1998, 151.
- 35 Velde V V & Lavie D, *Phytochemistry*, **21** (1982) 731.
- 36 Uma Devi P, *Indian J Exp Biol*, **34** (1996) 927.
- 37 Uma Devi P, Sharada A C & Solomon F E, *Cancer Lett*, **95** (1995) 189.
- 38 Uma Devi P, Sharada A C & Solomon F E, Akagi K & Tanaka Y, *Sensitiz Newslett (Japan)*, 01 July (1998) **8**.
- 39 Uma Devi P, Akagi Ostapenko V, Tanaka Y & Sugahara, *Int J Radiat Biol*, **69** (1996) 193.
- 40 Sharada A C, Solomon F E, Uma Devi P, Udupa N & Srinivasan K K, *Acta Oncol*, **35** (1996) 95.
- 41 Ganasoundari A, Zare S M & Uma Devi P, *Br.J Radiol*, **70** (1997) 599.
- 42 Chowdhary K & Neogy R K, *Biochem Pharmacol*, **24** (1975) 919.
- 43 Fungler A, *Arzheim Forsch*, **32** (1973) 923.
- 44 Shohat B, Kirson I & Lavie D, *Biomedicine*, **28** (1978) 18.
- 45 Bahr V & Hansel R, *Planta Med*, **44** (1982) 32.
- 46 Anbalagan K & Sadique, *J Curr Sci*, **50** (1981) 88.
- 47 Ghosal S, Lal J, Srivastava R, Bhattacharya S K, Upadhyay S N & Jaiswal A K, *Phytother Res*, **3** (1989) 201.
- 48 Luis J G, Echeverri F, Garcia F & Rajas M, *Planta Med*, **60** (1994) 348.
- 49 Sethi P D, Thiagrajan A R & Subramanian S S, *Indian J Pharmacol*, **2** (1970) 165.
- 50 Sethi P D & Subramanian S S, *Indian J Pharmacol*, **4** (1972) 30.
- 51 Sethi P D, *Indian J Pharm*, **38** (1976) 162.
- 52 Budhiraja R D, Sudhir S & Garg K N, *Planta Med*, **50** (1984) 134.
- 53 Syrov V N, Khushbaktova Z A & Vasina O E, *Khim Farm Zh*, **23** (1989) 610.
- 54 Al-Hindawi M K, Al-khafazi S H & Nabi M H A, *J Ethnopharmacol*, **37** (1992) 113.
- 55 Chada Y R, *The Weath of India, Raw materials* (Publication and Information Directorate, New Delhi, India) 1976, p. 580.
- 56 Sudhir S, Budhiraja R D, *Indian J Physiol Pharmacol*, **36** (1992) 127.
- 57 Budhiraja R D, Garg K N, Sudhir S & Arora B, *Planta Med*, (1986) 28.
- 58 Krishan P, Budhiraja R D, Arora B, Seth R K & Sudhir S, *Proc Forty-fifth Annu APPI Conf*, (1999) p. 601 A.
- 59 Bhattacharya S K, Satyan K S & Ghosal S, *Indian J Exp Biol*, **35** (1997) 236.
- 60 Tripathi Y B, & Shukla S, *Indian J Biochem Biophys*, **32** (1995) 308.
- 61 Ascher K R S, Nemny N E, Eliyahu M, Kirson I, Abraham A & Glotter E, *Experientia*, **36** (1980) 998.
- 62 Yamamoto R T & Frankel G S, *Ann Entamol Soc Am*, **53** (1960) 503.
- 63 Ascher K R S, Schmutterer H, Glotter E & Kirson I, *Phytoparasitica*, **9** (1981) 197.
- 64 Ascher K R S, Eliyahu M, Glotter E, Kirson I & Abraham A, *Phytoparasitica*, **12** (1984) 147.
- 65 Ascher K R S, Eliyahu M, Glotter E, Goldman A, Kirson I, Abraham A, Jacobson M & Schmutterer H, *Phytoparasitica*, **15** (1987).
- 66 Bates R B & Morehead S R, *J Chem Soc Chem Commun.*, (1974) 125.

- 67 Budhiraja R D, Sudhir S & Garg K N, *Indian J Pharmacol*, **27** (1983) 129.
- 68 Budhiraja R D, Sudhir S & Garg K N, *Indian J Pharmacol*, **16** (1983) 60.
- 69 Krishan P, Budhiraja R D & Sharan S, *Proc ICPT-21*, New Delhi, 1999, p. 118.
- 70 Schliebs R, Liebmann A, Bhattacharya S K, Kumar A, Ghosal S & Bigl V, *Neurochem Int*, **30** (1997) 181.
- 71 Bhattacharya S K, Kumar A & Ghosal S, *Phytother Res*, **9** (1995) 110.
- 72 Ghosal S, Kaur R & Bhattacharya S K, *planta Med*, **54** (1988) 561.
- 73 Ghosal S, Kaur R, Srivastava R, *Indian J Nat Prod*, **4** (1998) 12.
- 74 Bhattacharya S K, Goel R K, Kaur R & Ghosal S, *Phytother Res*, **1** (1987) 32.
- 75 Ramarao P, Rao K T, Srivastava R S & Ghosal S, *Phytother Res*, **9** (1995) 66.
- 76 Sharada A C, Solomon F E, Uma Devi P, Srinivasan K K & Udupa N, *Amala Res Bull*, **13** (1993) 13.