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Review Article

Development of Multiple Use *Mycobacterium indicuspranii* Vaccine, Meritorious Probiotics and Polyherbal BASANT for restoring Vaginal Health

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Abstract

Briefly described are 3 useful products resulting from basic research leads to undergoing toxicology, Phase I/II/III clinical trials and thereon to products transferred to industry. *Mycobacterium indicuspranii* (MIP) was developed as an Immunotherapeutic cum Immuno-prophylactic vaccine against leprosy. It has received the approval of Drugs Controller General of India (DCGI) and USFDA. It is transferred to industry and is available to the public.

MIP is a potent invigorator of immune responses. As adjuvant in an anti-hCG vaccine, it enhances considerably antibody titres. Used live or autoclaved, MIP is protective against tuberculosis and has no genetic restriction. As adjunct to MDT, it has superior cure rate of Category II 'Difficult to treat' tuberculosis patients.

Given intra-lesionally, MIP cures dramatically ugly ano-genital warts. It has preventive and therapeutic action against SP2/o Myelomas in BALB/c mice.Three meritorious strains *Lactobacilli: L.salivarius* TRF #30, *L. fermentum* TRF# 36 and *L. gasseri* TRF# 8 have been isolated and characterized. These have been passed on to industry. Also developed is a Polyherbal microbicide BASANT with a wide spectrum action on bacterial, fungal and viral genital pathogens including those resistant to drugs. Besides preventing the entry of HPV16, it eliminates HPV16 from infected cervical cells, restoring to normalcy the Pap smear.

Keywords: Adjuvant; Ano-genital warts; Myeloma; Tuberculosis; Vaginosis

Prologue

Any worthwhile advance in Biotechnology has to reach people via industry so as to benefit the users. Described below are a few products which have resulted from our work.

A Unique Immunoprophylactic Cum Immune-Therapeutic Vaccine Against Leprosy Endowed with Multiple Additional Applications of High Utility

We discovered a saprophytic, cultivable mycobacteria, which shares antigens with both *M. leprae* and *M.tuberculosis*. This mycobacterium coded as Mw is now defined by its gene sequence [1-3]. Being a hitherto new micro-organism, previously unregistered in International Depository, it has been named as *Mycobacterium indicuspranii* (MIP). Pran is my familiar name and NII, the National

Institute of Immunology where bulk of research was done on it under my leadership. (Figure 1) gives the electron micrograph and atomic force microscopic vision of MIP.

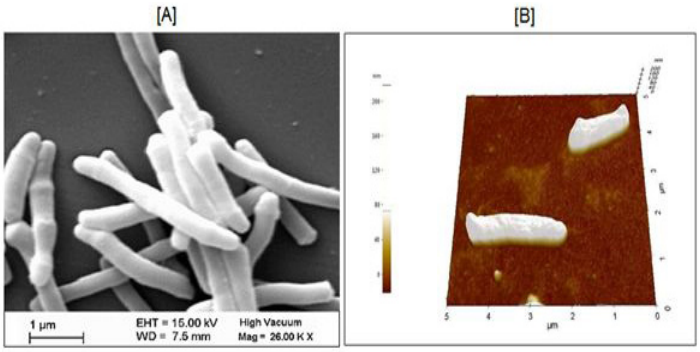


Figure 1: *Mycobacterium indicuspranii* (MIP). [A] Electron Micrograph [B] Atomic Force Microscopy Perspective.

MIP as adjunct to standard multi-drug regime expedites bacterial clearance and shortens the period of recovery of leprosy patients [4]. It converts upto 68% of Lepromatous Leprosy (LL) patients from lepromin negativity to lepromin positivity status. LL patients lack immunity to respond to *M. leprae* antigens and remain lepromin negative even after clearance of bacilli by drugs. Drugs kill bacteria but do not improve the immuno-reactivity of LL patients to *M. leprae* antigens. MIP has thus this unique ability to not only clear bacilli, but also rectify to a large extent the immune deficit in persons lacking it completely. Another notable feature of using MIP along with drugs is to attain complete recovery without residual blemishes. (Figure 2) shows few patients treated with MIP in addition to drugs.

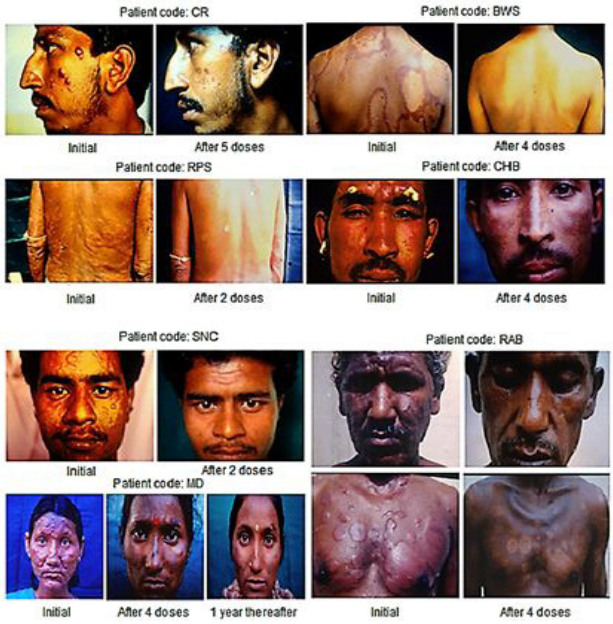


Figure 2: Some Representative Cases of LL/BL Multibacillary Patients Treated with MDT plus MIP (*Mycobacterium indicuspranii*).

MIP has received the approval of the Drugs Controller General of India (DCGI) and also of USFDA. It is at present the only vaccine of its type in the world. It has been passed on to industry. M/s Cadilla Pharma Ahmedabad, manufacture and make it available to public.

Additional Applications of MIP

Tuberculosis

Guinea pigs immunized with MIP do not fall victim to H37RV virulent strain of tuberculosis. MIP has been employed with good results for treatment of category II "Difficult to treat" tuberculosis patients. (Table 1) gives the results of one of these trials.

Treatment Description	Cured	Cured (%)
MIP + MDT (n=49)	48/49*	97.96
MDT alone (n=27)	21/27**	77.77
*One patient defaulter for 6 doses, sputum negative after intensive phase. ** Six patients-No effect of therapy		

Table 1: Outcome of the Additive Effect of MIP (Mw) in Comparison to MDT Alone for Therapy of Category II Tuberculosis Patients.

Furthermore, the relapse rate of patients treated with MIP, in addition to standard drugs, is far lower than those treated with drugs alone (Figure 3).

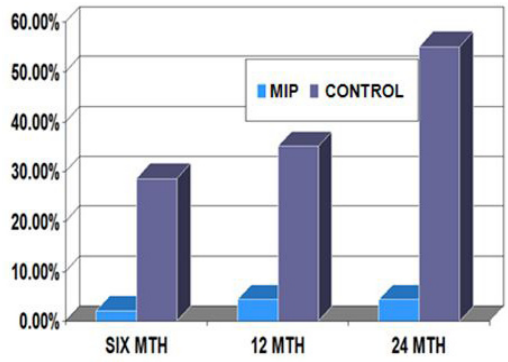


Figure 3: Relapse Rate of Category II Tuberculosis Patients After Treatment with MDT Alone or MDT+MIP.

MIP has several advantages over BCG for prophylactic immunization. It is active in both live and killed form, whereas BCG is inactive in non-live state.

BCG is protective in some countries, such as Uganda, but not in others, such as India. There appears to be genetic restriction on its immunization capability. (Figure 4) shows that BALB/c and C57BL/6 strains of mice are protected by both BCG and MIP, whereas C3H and CBA strains of mice immunized with BCG do not resist challenge with H37RV,but all mice immunized with MIP are protected [5].

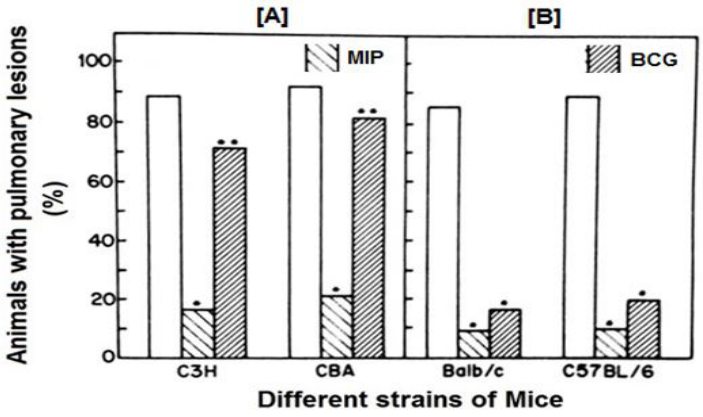


Figure 4: Effect of Immunization with killed MIP or live BCG on development of pulmonary lesions in C3H, CBA (Panel a) and in Balb/c, C57BL/6 (Panel b) strains of mice. Animals were immunized either with 107 heat-killed MIP s.c. or 104 live BCG i.v. Four weeks later, the animals were challenged with 107 *M. tuberculosis* H37Rvi.v. Four weeks' post challenge, the animals were killed and visible lesions in the lungs were recorded. The data plotted are the mean of results obtained from three sets of experiments with MIP and two sets of experiments with BCG, with n=7-10 animals per group. The data for the non-immunized group of animals are also presented (Blank bars).

MIP: A Potent Adjuvant

We are in process to develop a Birth Control Vaccine, directed against Human Chorionic Gonadotropin (hCG). Human Chorionic Gonadotropin (hCG) is not made by any organ of non-pregnant non-cancerous woman. That is why its appearance in urine /blood is a reliable test for diagnosis of pregnancy. It is the early embryo following fertilization of the egg that starts making HCG as observed by Bob Edwards [6]. Human Chorionic Gonadotropin (hCG) plays an important role in implantation of the embryo onto the endometrium resulting in onset of pregnancy. Antibodies binding with hCG prevent this process, hence prevent pregnancy. These do not interfere with normal reproductive functions of the woman. She ovulates normally and makes her sex hormones normally. Regularity of menstrual cycles is maintained with normal bleeding profiles. Vaccines against hCG made by us earlier have undergone Phase I/Phase II clinical trials in India and also in Finland, Sweden, Chile and Brazil, which have shown the safety and reversibility of the vaccine [7]. Antibodies above 50 ng/ml titre prevent pregnancy without derangement of menstrual regularity [8].

We have now developed a recombinant vaccine against hCG, which is amenable to industrial production. This vaccine, hCGβ-LTB, is fairly immunogenic in mice. MIP has been used as adjuvant in this vaccine. As shown in (Figure 5), inclusion of MIP as adjuvant enhances by several folds antibody titres against hCG [9].

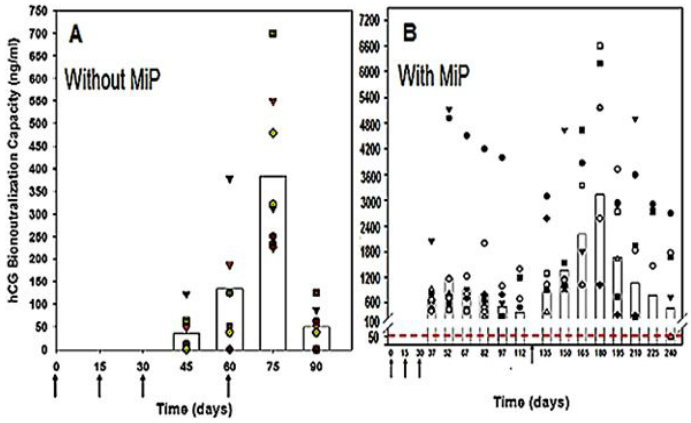


Figure 5: Antibody titres against Human Chorionic Gonadotropin (hCG). Symbols represent titres in individual mice, Bars give the geometrical mean titres [9].

Preventive and Therapeutic Action of MIP on Myelomas

Prof. Dipankar Nandi at the Indian Institute of Science, Bangalore has published highly interesting properties of MIP in curtailing the development of Myelomas caused by SP2/o cells in BALB/c mice. (Figure 6) is a summary of his findings reported elsewhere [10].

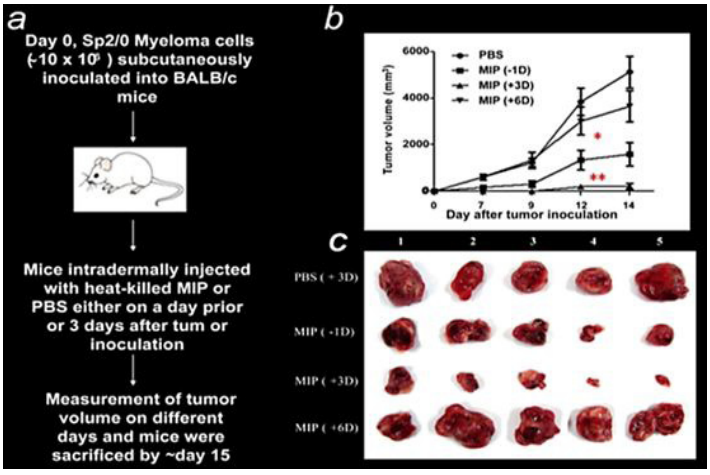


Figure 6: Preventive and Therapeutic Action of MIP Against SP2/O Myelomas in Mice.

Healing of Ugly Warts on Ano-Genital Region and Feet

Prof. Somesh Gupta at the All India Institute of Medical Sciences, New Delhi employs MIP intra-lesionally to cure warts on sperm and rectum (Figure 7-8) as well as on feet (Figure 9). These findings are reported in 2 publications [11,12].

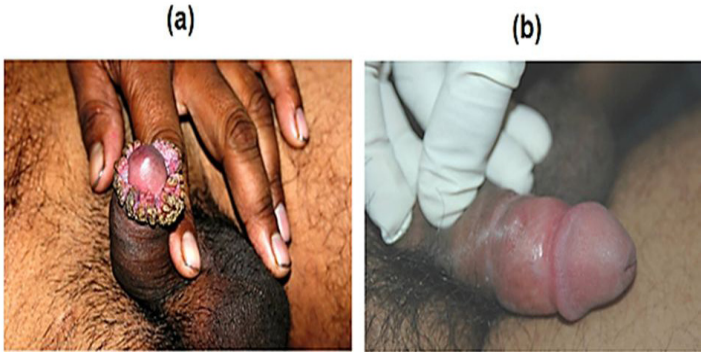


Figure 7: Healing by MiP of Ugly Ano-Genital Warts (A) Patient with Giant Condylomata(B) Full clearance of Lesions with Intralesional Immunotherapy with MIP.

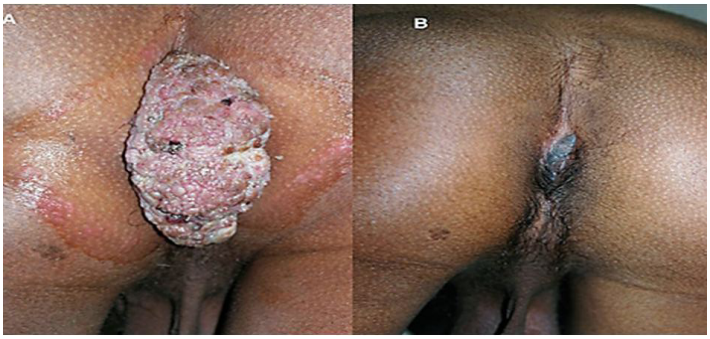


Figure 8: (A) Before treatment (B) After treatment with MIP.

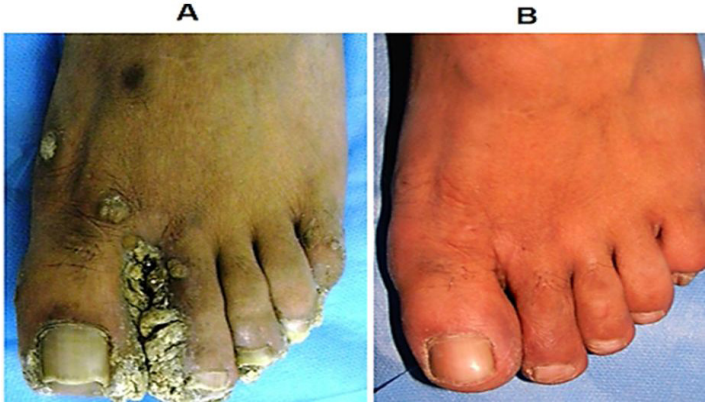


Figure 9: Cure by MIP of Warts on Feet. (A) Before Treatment and (B) After 5 months of Treatment with MIP.

Probiotics

A healthy human vagina has an acidic pH of about 4, whereas the pH of the rest of body of normal individuals is rigorously maintained at 7.4. The local acidic pH of vagina is caused and maintained by *Lactobacilli* resident in harmony with endometrial cells of the vagina. Lactobacilli make and secrete D- and L-Lactic acid in addition to Bacteriocins, and H_2O_2. Good strains of Lactobacilli have also the enzyme Arginine deiminase, which prevents the formation of foul odor derivatives.

We isolated 80 Probiotic strains from women with healthy vagina. These were characterized on the basis of Genus, Group and Species by specific PCRs along with Random Amplified Polymorphic DNA (RAPD) and 16s rDNAs. The findings are reported elsewhere [13]. The predominant species isolated were *L. gasseri, L. fermentum, L. salivarius, L. plantarum*). All bacilli were gram positive and catalase negative. They all made and secreted lactic acid. Many made also H2O2. It was observed that strains of even the same species differed in their capacity to make and secrete lactic acid [14]. Strains isolated from women with healthy vagina made twice as much D-lactic acid as those isolated from women suffering from Vaginosis (Figure 10).

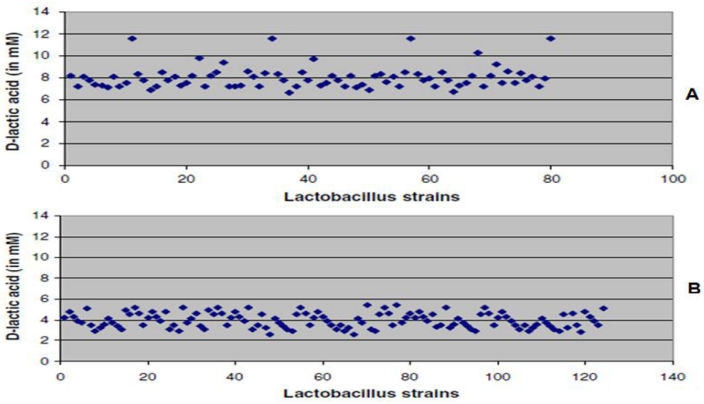


Figure 10: D-lactic acid Production by (A) 80 *Lactobacillus* Strains from Women with Healthy Vagina (B)124 Strains from Women Suffering from Bacterial Vaginosis [14].

On basis of their property of making and secreting high amounts of D-lactic acid, Bacteriocins, H_2O_2 and positive for Arginine deiminase, we selected 3 meritorius strains: *L. salivarius* TRF # 30,L. fermentum TRF # 36, and *L. gasseri* TRF # 8. These have been passed on to M/s Microbax Hyderabad for large-scale culture, lyophilisation along with stabilizers and packaging in easily dispersible cellulose capsules, which can be inserted in vagina. Their high acceptability by women and their ability to restore healthy vaginal pH has been established by appropriate clinical trial.

Polyherbal Formulation BASANT

Vaginosis is manifested as abnormal vaginal discharge, vaginal pH>5 and presence of aerobic, anaerobic microorganisms. It is a commonly occurring syndrome. Its incidence is 30% in women in urban localities [15], 50% in rural Maharastra [16] and 80% in slums of India [17]. It is not confined to India, 29.2 % of women are reported to suffer from it in USA [18].

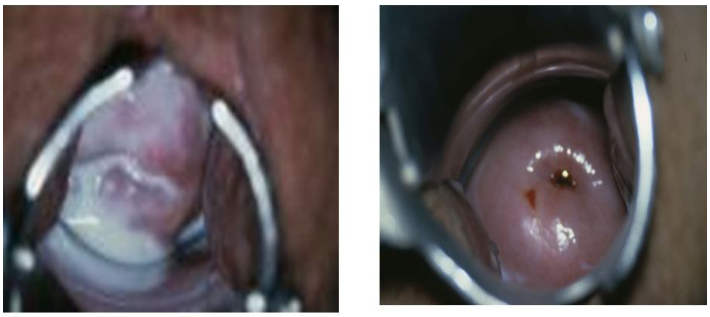
We developed a polyherbal formulation, named as BASANT, containing 95% pure Curcumin, purified extracts of Amla (*Emblica officinalis)*, Neem (*Azadirachtaindica*) leaves, and Aloe vera (*Aloe barbadensis*) together with pharmacopoeially approved

excipients. BASANT has wide spectrum action on sexually transmitted pathogens. It inhibits *Neisseria gonorrhoeae*, including strains resistant to Penicillin, Tetracycline, Nalidixic acid and Ciprofloxacin [19]. It has pronounced inhibitory action against *Candida glabrata*, Candida albicans and *Candida tropicalis* isolated from women with vulvovaginal candidiasis, including isolates resistant to azole drugs and amphotericin. It has inhibitory action on free as well as cell infected *Chlamydia trachomatis* [20]. BASANT was found to be totally safe as per USFDA recommendations on rabbit vagina after application for 7 consecutive days and twice daily for 3 weeks.

BASANT inhibits HIV. It prevents the entry of CCR5 and CXCR4 tropic HIV 1 lab-adapted strains and primary isolates belonging to different clades [21]. BASANT inhibits the entry of HPV16 in Hela cells [19]. Furthermore, it has the unique property of eliminating HPV16 from the infected cervical cells on path of progression to Carcinoma of Cervix at early stages when the virus is not yet integrated in host genome. In a trial conducted in Aligarh, Uttar Pradesh, in 11/11 women whose cervical cells were infected with HPV16, the virus was eliminated from infected cells, restoring normal Pap smear [22].

BASANT is highly effective in providing relief to women suffering from recurring episodes of vaginosis. (Figure 11) gives typical results obtained by using BASANT.

(a) Relief from abnormal vaginal discharge



ON ENROLLMENT AFTER TREATMENT

(b) Disappearance of Clue cells:

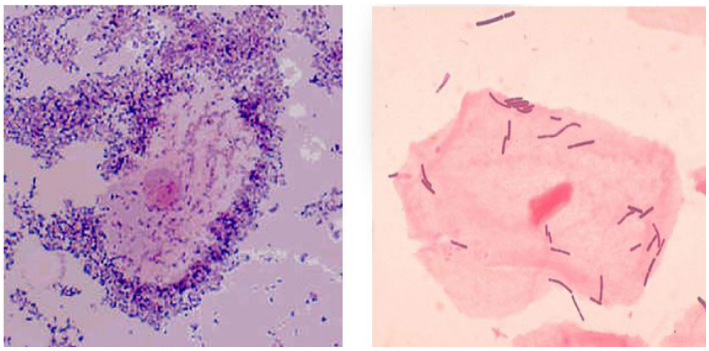


Figure 11: An Illustrative Representation of Typical Woman Receiving the Treatment with Combination of BASANT plus Probiotics.

A combination of BASANT along with the 3 selected meritorious Probiotics, cures almost 95% of women suffering from Vaginosis (Table 2). This is the highest efficacy achieved by any treatment in the world literature. Antibiotics cure only up to 65% of such patients.

Treatment given to 20 women in each group	Improved (%)	P value Comparison with Placebo	P value Comparison with BASANT+Probiotics
Probiotics	13 (65%)	P<0.001	P = 0.04
BASANT	14 (70%)	P<0.001	P = 0.09
BASANT+ Probiotics	19 (95%)	P<0.001	-
Placebo	1 (5%)	-	P<0.001

Table 2: Summary of Results of Treatment given to the four Groups of Women Suffering from Recurrent Vaginosis [23].

Summary

Recapitulated above is our work on Problems of relevance. In each case, the work was followed up by developing potential solutions. After defining the nature of immune deficit in leprosy patients, a vaccine was developed based on a cultivable non-pathogenic atypical mycobacterium whose gene sequence has been determined. It has been named as *Mycobacterium indicuspranii* (MIP). MIP along with standard drugs expedites bacterial clearance and shortens the period of blemish free recovery. This vaccine is approved by the DCGI (India) and USFDA. It is passed on to Industry and is available to public.

MIP prevents H37RV induced tuberculosis in guinea pigs. Unlike BCG, it has no genetic restriction for response. It is active in both live and killed form. Used as adjunct to standard drugs, it improves significantly the cure rate of Category II 'Difficult to treat' tuberculosis patients. MIP heals dramatically ugly ano-genital warts and prevents SP2/o myelomas in mice.

Also, passed on to Industry are 3 selected meritorious Probiotics strains for vaginal use, which are high secretors of lactic acid and prevent foul odor derivatives. At final stage of going to In-

dustry is a Polyherbal microbicide BASANT, which inhibits *N.gonorrhoeae,C. trachomatis*, HIV, HPV, Candida'sincluding drugs resistant strains. It eliminates HPV-16 from infected cervical cells restoring normal Pap smear.

Acknowledgements

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