Background (from application):
AIDS, tuberculosis (often associated with AIDS) and malaria are resulting in the death of millions of people in southern Africa, such that the hope and opportunity of a social, economic and cultural renaissance is being severely threatened. Needless to say this prognosis is true for much of the rest of Africa.

As is true for societies the world over, the peoples of southern Africa have a rich history of reliance upon indigenous plants for a wide variety of traditional medicines. Indigenous herbal medicines have been used for centuries as therapeutics and are very important components of South Africa’s Indigenous Knowledge Systems (IKS). It is estimated that 80% of South Africans continue to rely upon herbal medicine for their primary health care. While the use of traditional herbal medicines has been marginalized, stigmatized and discredited as primitive ritual and witchcraft by Western medicine and many years of colonialism and then apartheid, this marginalization and stigmatization is being reversed in South Africa. Indeed this marginalization is also being reversed in the United States where there is rapidly increasing use of indigenous medicinal plants and other botanicals. The increasing demand for South African medicinal plants is increasing the collecting pressure on wild populations of medicinal plant species, threatening the existence of many of these species before their potential has been scientifically evaluated – either as herbal medicines or as sources of medicinally useful compounds.

South Africa has a dual healthcare system with approximately 22,000 allopathically trained doctors in the public health system. These doctors stand in the midst of the HIV/AIDS epidemic, with the accompanying multi-drug-resistant secondary infections, tight hospital budgets, a limited supply of drugs and constantly maturing equipment. Within this environment, these physicians serve approximately 44 million people, who speak 11 different languages and hold a diversity of spiritual and religious views. The second arm of the dual healthcare system in this complex and challenging health care environment comprises of the reality that some 80% of South Africans consult with over 200,000 traditional doctors/healers in the informal healthcare sector, seeking safe, effective and affordable medicines to modulate their immune systems and to combat AIDS and its secondary infections. These traditional doctors (Inyangas and Sangomas) utilize phytotherapies derived from one of the most botanically diverse environments in the world.

Southern Africa has the richest temperate flora in the world and encompasses a rich floristic diversity of approximately 24,000 taxa (species and infraspecific taxa) and includes more than 10% of the world’s vascular plant flora known to humankind on less than 2.5% of the earth’s land surface. Traditional doctors use over 3,600 species as medicines. Of these, 350 species are traded as medicinal plants with an estimated market valued at around $350 million. This industry creates significant employment for a public that is searching for a sustainable means to live.

With the use of these two healthcare systems, the allopathic as well as the traditional healers, South Africa has a dual healthcare system with patients traversing both the
conventional and traditional healing streams, dependent upon the nature of their condition and socio-economic status. However, this is less than optimal since individuals often may not disclose that they have consulted both western and traditional doctors, and that they may be taking medicines that may negatively impact one another from both practitioners.

South Africa has recognized the critical need for the legal recognition of traditional doctors and their indigenous knowledge about health and healing systems. The South African government is highly interested in exploring IKS and believes that the traditional healers could be powerful partners with allopathic doctors in jointly treating patients for HIV/AIDS, secondary infections and a variety of other disorders. In addition, the traditional healers have expressed a strong desire to be regularized in order to eliminate the charlatans from amongst their ranks and to protect the honor and dignity of their ancestors and traditions.

Leaders in South Africa have taken a number of key initiatives to deal with the unique situation of African Traditional Medicines. The National Drug Policy (1996 - www.doh.gov.za/docs/policy/drugsjan1996.pdf) recognizes the potential role and benefits of African Traditional Medicines in the National Health System. This policy is aimed at investigating the use of effective and safe traditional medicines at a primary level and further addresses (1) the investigation of traditional medicines for efficacy, safety, quality with the aim to incorporate their use in the national healthcare delivery system; (2) registration and control of marketed traditional medicines; and (3) provisions of for the establishment of a National Reference Center for African Traditional Medicines for purposes of gathering harnessing and synthesizing information to promote, regulate and register African traditional medicines of plant origin. These policies have further been enhanced by the Traditional Health Practitioners Bill (www.pmg.org.za/docs/2003/appendices/031022b66-03.pdf) through which the Interim Traditional Health Practitioners Council of South Africa will be established; to provide for a regulatory framework to ensure the efficacy, safety and quality of traditional health care services; to provide for the management and control over the registration, training and conduct of practitioners, students and specified categories in the traditional health practitioners profession; and to provide for matters connected therewith.

The South African government took a significant step in 2003, when the Minister of Health, Dr. Manto Tshabalala Msimang, with the support of the Medical Research Council (MRC: see www.mrc.ac.za), Medicines Control Council (MCC: see www.mccza.com) and the Council for Scientific and Industrial Research (CSIR: see www.csir.co.za), launched the National Reference Centre for African Traditional Medicines (NRCATM). The goal of NRCATM is to advance the contribution of African traditional medicines to the health and well being of people of South Africa and the region, by promoting scientific validation of these remedies, which in turn will allow the production of high quality, safe medicines based on the indigenous phytotherapies. The World Health Organization (WHO) is committed to partnering with NRCATM to promote the use of safe, effective and affordable traditional medicines.

South Africa has moved a great deal toward recognizing traditional healers, their knowledge and healing ways, as being integral partners in the formal healthcare system required to meet the extreme challenges of the new millennium.

With such a rich heritage, South Africa has much to offer the world in the documentation and study of its indigenous knowledge systems and the practices of its traditional healers. Furthermore, in contrast to much better known traditional healing practices, such as the Ayurvedic and those of Asia, little is known in the Western world about the African phytotherapies that have been traditionally used for centuries. There is much greater
opportunity for significant new discoveries of valuable therapies, particularly as South Africa is home to such phenomenal botanical diversity.

In 2003 Dr. William Folk (UM-C Assoc. Dean, SOM) successfully spearheaded the compilation of a proposal to the NIH for a planning grant for the formation of The International Center for Indigenous Phytotherapy Studies (TICIPS), an International Center for Research on Complementary and Alternative Medicine. This proposal was funded by the NIH.

In 2005 a TICIPS Phase II proposal was submitted to the NIH (Folk, PI). This proposal was funded and is currently under review. Currently TICIPS is an international collaboration between the University of Missouri-Columbia, the University of the Western Cape, the University of KwaZulu-Natal (previously Univ. of Natal) and the Missouri Botanical Garden, St. Louis.

TICIPS has the following as central missions:

a) to create a collaborative environment of trust, open communication and research;
b) to support scientifically rigorous and ethical studies of African phytotherapies and healthcare systems;
c) to train scientists to conduct the highest caliber research in complementary and alternative medical practices (CAM);
d) to promote conservation of plant biodiversity and protection of indigenous knowledge;
e) to communicate evidence for the safety and efficacy of indigenous phytotherapies and CAM to healthcare providers and the public;
f) to advance the health and well-being of the African and American peoples.

There are four research projects in the TICIPS Phase II proposal:

- Research Project 1: A Phase I/II clinical trial of *Lessertia frutescens* (L.) Goldblatt & J.C. Manning (syn. *Sutherlandia frutescens* (L.) R. Br.) phytotherapies in HIV-infected adults;
- Research Projects 2 & 3: Preclinical studies in rodents using *Artemisia afra* Jacq. ex Willd. phytotherapies in the treatment of cervical cancer and tuberculosis, respectively;
- Research Project 4: A Phase 1 safety clinical trial of *Artemisia afra* phytotherapies in healthy adults.

The University of Missouri partnership with the University of the Western Cape, South Africa and the Missouri Botanical Garden provided an ideal foundation for the collaborative research program of the TICIPS Phase I grant. The TICIPS Phase II research program is considerably expanded both within the USA and in South Africa, the latter expansion involving collaborations with colleagues at the Nelson R. Mandela School of Medicine at the University of KwaZulu-Natal, South Africa.
Purpose of visit to South Africa (UWC & UKZN)

Objectives (outcomes):
1. Relationship building;
2. Plant Systematics and phytochemical research on *Artemisia afra* populations to document the presence and variability of the glandular trichomes and associated terpenoid phytochemistry of material collected in the Cape and KwaZulu-Natal provinces of South Africa.

The plant systematics research will be conducted jointly by Mr. Weitz (UWC) and Dr. Vincent (UM-C) via the Dunn-Palmer Herbarium. Any exchanges of research material will be arranged between the Curators of the herbaria on the respective campuses, as needed.

The phytochemical profiling of the collected material will be performed by Dr. Mabusela in the Department of Chemistry, UWC. Additional phytochemical profiling may be conducted by Dr. Larry Walker’s lab. (NCNPR), Univ. of Mississippi, USA.

It is envisaged that one or more publications should result from this collaborative research.

This research and relationship building experience will provide further potential to leverage future external support.

Collaborations will be with the following colleagues:
1. Mr. Frans Weitz – Department of Biodiversity & Conservation Biology, UWC;
2. Dr. Wilfred Mabusela – Department of Chemistry, UWC;
3. Dr. Quinton Johnson – Director, South African Herbal Science and Medicine Institute, UWC;
4. Dr. Jeremy Klaasen – Researcher and administrator in the South African Herbal Science and Medicine Institute (SAHSMI), UWC;
5. Dr. C. Nceba Gqaleni, Deputy Dean, Nelson R. Mandela School of Medicine, University of KwaZulu-Natal. Dr. Gqaleni will introduce me to leaders amongst the Traditional Healer community in Durban who in turn will facilitate my field collecting of *Artemisia* material in KwaZulu-Natal;
6. Mr. James Hartzell, Nelson R. Mandela School of Medicine, University of KwaZulu-Natal and a member for the Traditional Healers Core of the TICIPS research program;
7. One or more traditional healers who are collaborators on the TICIPS project and members of the Traditional Healers Core

Scientific Research:

Overview: Systematics and phytochemical research on the genus *Artemisia* in South Africa

Three species of *Artemisia* (family: Asteraceae (daisies)) are recorded in the flora of southern Africa (*A. afra* Jacq. ex Willd., *A. vulgaris* L. & *A. absinthium* L.). Only *A. afra* is indigenous to southern Africa and is widely used in traditional medicine. Research on the terpenoid phytochemistry of *A. afra* from various wild populations has revealed the existence of chemotypes. These chemotypes reflect the presence of divergent genotypes amongst the wild growing, open pollinated populations of *A. afra* (Graven *et al.*, 1990). This finding is significant because traditional healers could be prescribing *A. afra* phytotherapies from a
range of these chemotypes, with resultant variation in success of treatment. Furthermore, this finding is significant because some of the research projects of the TICIPS Phase II research program are heavily focused on the use of *A. afra*. Consequently, it is essential that any variation in the phytochemical composition of the plant material of *A. afra* that is to be used in the research projects, be known.

Research on the phytochemistry of *Artemisia annua* L., a putatively close relative of *A. afra* which is indigenous to Asia, revealed the presence of a chemotype which did not contain the antimalarial compound artemisinin. Further investigation revealed that this artemisinin-free chemotype did not possess the glandular hairs that typically cover the leaves of *A. annua* (Duke *et al.*, 1994). The glandular hairs are the site of synthesis and storage of artemisinin, a sesquiterpene compound. This documentation of a glandless biotype in *A. annua* is of considerable significance and is part of the motivation behind this proposal.

Preliminary studies have identified the terpenoids, artemin, arsubin and tanacetin to be present in *A. annua* material. However, significant quantities of artemisinin in extracts of *A. afra* (comparable to those present in *A. annua*) have not been detected (pers. comm. W. Mabusela, unpublished). Further preliminary data demonstrates that extracts of *A. afra* selectively limits the growth of virulent and avirulent mycobacteria *in vitro* using several different experimental endpoints for measuring bacillus growth and viability. Furthermore, the water-soluble artemisinin derivative, artesunate, inhibits the *in vitro* growth of several cancer cell lines, with leukemias and colon cancer cell lines being the most sensitive (1-3 μM range) and non-small cell carcinomas of the lung being the most resistant (25 μM range). Cervical cancer cell lines have not been evaluated.

Although preliminary investigations have not detected artemisinin in *A. afra*, that may be due to problems with its stability. However, the other terpenoids, artemin and arsubin appear to be compounds with similar biological activity and are likely to prove of interest.

While *A. afra* is reportedly the only species utilized by traditional healers in South Africa (notwithstanding the occurrence of *A. vulgaris* and *A. absinthium* in the region) no documentation has been found which reports comparative studies on material of *A. afra* from the Cape Province and from the KwaZulu-Natal Province. It is imperative for both the anticipated TICIPS research and the ongoing utilization of *A. afra* material by Traditional Healers, that information on the presence and variation of terpene chemistry (especially that of artemisinin) be obtained. Furthermore, no information has been located which confirms that only *A. afra* plant material is collected and used by traditional healers in the Cape and KwaZulu-Natal Provinces.

**Research questions to be investigated:**

1. Is the variation in terpenoid chemistry in *A. afra* associated or correlated with the variable presence of glandular trichomes on the leaves of *A. afra*?
2. Is there a glandless biotype of *A. afra*?
3. Is the morphology and phytochemistry of material of *A. afra* collected and utilized by traditional healers in the Cape Province comparable to that of material similarly collected and utilized in the KwaZulu-Natal Province?
4. What is the terpene (monoterpene and sesquiterpene) profile in *A. afra* and how does it vary amongst one or more select populations of *A. afra* in the Cape and KwaZulu-Natal provinces?
5. Is material of *A. afra* consistently collected by traditional healers or is material of *A. vulgaris* and *A. absinthium* accidentally mixed with material of *A. afra*?
A core hypothesis of this research is the following:

_Variation in the terpenoid chemistry in populations of _A. afra_ is correlated with the variable presence of glandular trichomes on the leaves._

**Research to be conducted (overview of methodology):**

1. Field collecting of _A. afra_ in the Cape Province. Areas to be visited would be the farm in the Montagu region, in the Western Cape, where _A. afra_ is cultivated and from where material was collected in April 2005 for TICIPS research; other select localities in the southern Cape from which material of _A. afra_ is collected by traditional healers;

2. A few samples of _A. afra_ will be sought for purchase from traditional healers in Cape Town – this facilitate evaluation of the consistency of the identity of the material being sold to consumers;

3. Field collecting of _A. afra_ in KwaZulu-Natal from one or more locations where traditional healers collect material.

4. Voucher specimens would be made of all collected material. Vouchers will be curated at the UWC herbarium and at the Dunn-Palmer herbarium, UM-C.

5. Specimens from the collecting localities in the Cape and KwaZulu-Natal will be studied with respect to a select suite of morphological and micromorphological and anatomical features (characters) including the presence of glandular trichomes. This will involve both light microscopy and scanning electron microscopy studies at UWC and/or UM-C. This phenotype data will be analyzed using numerical phenetics software which will facilitate the recognition of discontinuities between material from the different collecting localities and within collecting localities.

6. Samples of the same specimens that will be used for the morphological numerical phenetics study will be used in a study of the phytochemical profile of terpene compounds. This material will be extracted in the Department of Chemistry, UWC, following methodology that is similar to that used by Duke _et al._ (1994) and/or includes analysis by GLC/GC-MS in the lab of Dr. Wilfred Mabusela using a non-polar HP-5MS 5% phenylmethylsiloxane capillary column utilizing helium as carrier gas and a variety of temperature programs which may vary from sample to sample. Confirmation of structures will be facilitated by the MS component and by matching retention times against standards. Phytochemical data will be compared with the data provided by Graven _et al._ (1990) and Duke _et al._ (1994). This phytochemical data will also be included in the numerical phenetics study;

7. If considered necessary a portion of the same samples of plant materials analyzed by Dr. Mabusela of UWC will be analyzed by Dr. Larry Walker’s lab. (NCNPR), Univ. of Mississippi, USA.

A plant collecting permit for collecting in KwaZulu-Natal has been obtained. An application for a similar permit for collecting in the Cape Province is being reviewed by the CapeNature permit granting authorities. Should this application not be successful the collecting will be done under the permit of Mr. Frans Weitz.

**Schedule:**

March 24: Departure from St Louis (Delta 1202; SAA 7798) for Johannesburg, RSA
March 25: Connecting flight to Pietermaritzburg, KwaZulu-Natal (SAA 8038)
March 25-27: Visit with parents-in-law in Howick, KwaZulu-Natal
March 27: Collect car-hire vehicle in Pietermaritzburg
March 28: Depart for UKZN with short visit with Dr. Mark Laing, UKZN-Pietermaritzburg – Dr Laing conducts research on the cultivation of medicinal plant species with Mr Hartzell (graduate student).
March 28-April 3: Meet Dr. Gqaleni and Mr Hartzell at UKZN and traditional healer representatives of the Traditional Healers Core (Relationship-
April 04: Depart Durban for Cape Town (SAA 627)
April 04-10 Meet with and work with UWC collaborators and other TICIPS representatives (Relationship-building & research)
April 11 Depart Cape Town for St Louis (SAA 342, 209; Delta 7081)

Post-visit collaboration – May 2006 – January 2007: Continue with research to answer the research questions; synthesis of data; preparation of manuscript for publication. Proposed journal to be decided upon after consultation with colleagues in RSA.

<table>
<thead>
<tr>
<th><strong>Budget for 2006 Research Visit to UKZN &amp; UWC - Dr Leszek Vincent</strong></th>
<th>$$$</th>
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</thead>
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<tr>
<td><strong>Air Travel</strong></td>
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<td>Multiple destination air fare: STL to Johannesburg, to Pietermaritzburg; Durban to Cape Town; Cape Town to STL (S2481 Expedia quotation)</td>
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<td><strong>Room/board</strong></td>
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<td>KZN B&amp;B (R250 x 5 nights = R1,250/6) = $209</td>
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<td>KZN field work - accomm. (R250x2 nights =R500/6) = $84</td>
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<td>KZN Food $25/day (8 days) = $200</td>
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<tr>
<td>CPT - City Lodge Hotel R450 x 7 nights = R3150/6 = $525</td>
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<td>CPT Food $25 x 8 days = $200</td>
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<td><strong>Ground transportation - car hire</strong></td>
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<td>KZN: R140 (hire) + R100 (fuel)/day x 9 days = R2160/6 = $360</td>
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<td>CPT: R130 (hire) + R100 (fuel)/day x 7 days = R1610/6 = $269</td>
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<td>MO Express (roundtrip)</td>
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<td><strong>Personnel</strong></td>
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<td>KZN field guide: Accommod. (R250 x 2 nights = R500/5 = $84) + Food ($25 x 3 days = $75) + Compensation (R400 x 3 days = R1200/6 = $200). Total = $359 (estimate)</td>
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<td><strong>Materials/printing</strong></td>
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<td>UWC lab consumables</td>
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<td>Scanning Electron Microscopy/Photomicroscopy @ UM-C</td>
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<td>Handheld GPS tool for recording localities of plant collecting</td>
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<td>220-110V power converter + RSA socket adaptor</td>
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<td><strong>Communications</strong></td>
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<td>Mailing herbarium material to MO</td>
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<tr>
<td><strong>Other expenses</strong></td>
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<tr>
<td>Contingency funds (use in RSA) and purchase of plant collecting data from PRECIS database, South African National Biodiversity Institute</td>
<td>200</td>
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</tbody>
</table>
Contingency funds - additional phytochemical profiling by Larry Walker's Lab. (NCNPR), Univ. of Mississippi, USA)

References:


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**Report:**

As this research involved visits to the provinces of KwaZulu-Natal and the Western Cape the report will present information following the activities conducted in these two provinces.

**KwaZulu-Natal component:**

Some of the visit focused on developing relationships with colleagues in the Nelson Mandela School of Medicine on the Durban campus of the University of KwaZulu-Natal who are associated with the TICIPS research program. Contacts with colleagues on the Pietermaritzburg campus of the University of KwaZulu-Natal were mostly based on my long-term association with these colleagues (when I lived in Pietermaritzburg) and my desire to network with them about the goals of the TICIPS research program

**A. Relationship building and Research Activity—Durban:**

1. Contributed to relationship building with Dr. Nceba Gqaleni (acting Dean of the Medical School) and Mr. James Hartzell. Both are participants in the Traditional Healers Core of the TICIPS program.

2. Provided a copy of a Memorandum of Understanding (MOU) that I had compiled regarding my need to use GPS equipment to record the specific locality information of plant collecting sites. Concern had been raised by the traditional healers about my use of this technology, presumably because they did not want their collecting sites to become public knowledge via my GPS locality data becoming public. The MOU stresses the perspective that while GPS data is now the normative requirement of plant collecting permits, this data would not be available to the general public. There is considerable international precedence for the protocol outlined in the MOU. A copy of the MOU is attached.

3. Nceba and James had difficulty making contact with one of the traditional healers regarding collecting material of *Artemisia afra*. Problems were overcome and James coordinated a field trip to the Pinetown area (half an hour East of Durban) to meet with two traditional healers, Mrs Gamede and
Mr. Mkize. Mrs Gamede and Mr Mkize guided James and myself to a rural residential location where local inhabitants harvest wild-grown plants of *Artemisia afra*. Gamede and Mkize were very helpful in enabling James and myself to talk to the gatherers of the plant material. I used the opportunity to ‘teach’ all present about the physical process of plant collecting using a plant press, labeling, record keeping, GPS locality data handling. Voucher specimens and small bulk samples of plant material for phytochemical screening were obtained. Further information on the location is secured to protect this natural resource. Further information can be obtained from me or one of the participating herbaria (University of the Western Cape and Dunn-Palmer Herbarium, UM-C). I had purchased a set of GARMIN topographic maps for southern Africa. Having this topographic information was very helpful in homing in on potential good collecting sites that could have *A. afra*. This tool was very helpful during a subsequent all-day fieldtrip in the foothills of the Drakensberg Mountains.

4. I had hoped to be able to visit more collecting sites with *A. afra*, frequented by traditional healers, but the visit to the location outside Pinetown was the only opportunity. However, as *A. afra* is relatively common in KwaZulu-Natal, I was able to sample some of the diverse habitats/locations occupied by this species.

5. Provided the two plant gatherers with a gratuity as well as Mrs Gamede and Mr Mkize (the latter as they had to close their businesses during the fieldtrip. Also provided Mrs Gamede with a copy of the booklet “Zulu Botanical Knowledge” (previously obtained from the Natal Herbarium).

6. Dr Gqaleni and I drove to the Umlazi area, South of Durban, to visit with Mr M.P. Cele, a longstanding traditional medicine practitioner. We met with Mr Cele in his business premises (Store/shop). A detailed overview of the business practice and patient interest in this traditional medicine practice was provided by Mr Cele. Mr Cele also cultivates some of his own medicinal plant material at his Kumba Nature Reserve in Engonyameni.

7. During my stay at the Bed and Breakfast accommodation in Durban (“Hunters Green”, www.huntersgreen.co.za) I had valuable conversation with Mrs Jill Kruger (social anthropologist) of Jill Kruger Research and Film Production. She drew my attention to the “Deadly Myths” CD/DVD production. This film, made by Jill’s company for HIVAN on the UKZN-Durban campus, explores many of the urban myths surrounding HIV/AIDS and its transmission in South Africa. A copy of the CD/DVD was purchased from HIVAN for viewing amongst the TICIPS collaborators in USA. Mentioned Ms Patience Koloko, the President of the Traditional Healers Association in KwaZulu-Natal.

8. Visited the Natal Herbarium (NH) a facility of the South African National Biodiversity Institute. Reviewed their collection of *Artemisia afra* in preparation for a field-trip. Records were made of herbarium label information for coastal, midlands, uplands, Zululand, Lesotho, Cape/Transkei regions.

9. At the NH I met with Mr. Mkhipheni A. Ngwenya of the Zulu Botanical Knowledge Project. Amongst other things he affirmed the challenge posed by Zulu (iSizulu) plant names differing as one goes from the south coast of KwaZulu-Natal to the north coast. He also mentioned the cultural difficulty/reluctance amongst traditional healers to accept cultivated plant
material over wild harvested material. It would be interesting to conduct comparative phytochemical research on cultivated vs wild harvested material to explore this problem area.

10. Met with Dr. Neil Crouch in the Natal Herbarium. Discussed:
   a. Draft regulations on bioprospecting, access and sharing – not public yet but will be made public in due course for public comment (visit www.deat.gov.za). There are warning about the severe impracticability of this bill (where each taxon would require a collecting permit and MTA and Minister's consent and benefit sharing agreement—before work can be conducted. This could shut down access to medicinal plant researchers even within South Africa.
   b. Dr. Crouch is involved with the "Novel Drug Development Platform (NDDP –www.sahealthinfo.org/noveldrug/index.htm) – reported quite a few new screening 'hits';
   c. Talked about Dr. Delcon (sp?) (Dept. of Surgery, Univ. of Cape Town)—interested in immune modulation and 4-5 other disease areas;

B. Relationship building and Research Activity—Pietermaritzburg:

1. Courtesy visit with Prof. Hannes van Staden (Chairman of the Department of Botany, now retired), School of Biological and Conservation Sciences, UKZN; Director of Research Center for Plant Growth and Development. This visit was a follow-up from considerable e-dialogue (via email) with Prof van Staden in connection with work on *Hypoxis hemerocallidea* and his possible involvement in the TICIPS research project (TICIPS phase I). Discussed the TICIPS project (provided copy of TICIPS poster), my research visit plans. Prof van Staden was critical of some research groups "reinventing the wheel" by conducting research which his Center has already published.

2. Courtesy visit with Dr. Trevor Edwards, curator of the Herbarium (NU), School of Biological and Conservation Sciences, UKZN; Reviewed of herbarium specimens of *Artemisia afra*, especially those collected in KZN, to retrieve collection locality and habitat preference information in preparation for my field-work. Dr. Edwards was in the process of emigrating to Australia.

3. Several visits with colleagues at the Institute of Natural Resources (INR), associated with UKZN in Pietermaritzburg:
   a. Jennifer Mander – CEO of INR; provided copy of the following booklets:
   b. Dr. Chris Dickens – Researcher;
   c. Fonda Lewis – Contacts with Black Economic Empowerment (BEE) group that will be manufacturing botanicals via a new company in Isipingo (N-coast); Introduced me to PlantBio (National Innovation Centre for Plant Biotechnology – www.plantbio.org.za.
4. Met with Dr. Mark Laing (Chair of Plant Pathology and Director, African Centre for Crop Improvement), UKZN. This was a follow-up visit following email contact associated with exploration of a joint research program between MU and Dr. Laing, involving medicinal plant species via his ‘Crop Improvement Centre’. This visit was also motivated by exploration of interdisciplinary contact between colleagues at MU and UKZN and what is still needed to promote such interaction.

   a. Dr. Laing affirmed that MOU’s between Deans on different campuses tend to struggle at the implementation phase. Much better success is achieved via bottom-up collaboration, which in turn is supported by Deans on the different campuses. Dr. Laing cited the excellent working example between a Texas university and the Department of Grassland Science at UKZN—forged through a bottom-up approach. Texas students regularly conduct some of the coursework/fieldwork at UKZN.

   b. Dr. Laing has a leading biocontrol lab. and he’s keen to collaborate with colleagues in the USA regarding testing plant pathogens that are very close to human pathogens—but provide a much cheaper form of screening.

   c. Discussed the role of Near Infrared screening (NIR, e.g. FOSS-NIR) for rapid screening of samples and that it is fast and non-destructive and could be helpful in phytochemical screening of A. afra after calibration.


5. Met with Dr. Douglas Wilson MBBCh/MD at Edendale Hospital (TICIPS collaborator). Provided him with copy of TICIPS poster (printed & electronic) which he could use for education—much appreciated. Dr. Wilson introduced me to Pat, Langa and Zanele—three of Dr. Wilson’s assistants who will be working on the Sutherlandia study of TICIPS (provided copy of poster).

6. Following information provided by Dr. Nceba Gqaleni (UKZN, SOM) contact was made with Dr. Maria de Figueiredo (Analytical Services) of the KwaZulu-Natal Department of Agriculture and Environment Affairs in Cedara (outskirts of Pietermaritzburg). Dr. Figueiredo is researching the phytochemistry of Artemisia afra as this species is entering into cultivation by farmers who are supplying material for phytochemical screening. She reported on the presence of two chemotypes which appear to be genetically rather than environmentally produced. Provided me with copy of her PowerPoint presentation which included this topic. She suggested I make contact with Dr. Jim Simon (Rutgers Univ.) as Jim is also working on A. afra (searching for the presence of artemisinin, which has not been found yet). [I’m already in contact with Dr. Simon]

7. Made contact with Brian Abraham, the manager of the Silverglen Medicinal Plant Nursery (part of the Silverglen Nursery adjacent to Chatsworth,
Durban). A student/intern at the SANBI Natal Herbarium was my guide/navigator to reach the nursery. Purchased four (4) plants of Artemisia afra for cultivation at UWC and possible comparative phytochemical screening if needed/desired. These plants were subsequently given to Dr. Jeremy Klaasen who was going to give them into the care of the greenhouse manager at UWC.

8. Several very enjoyable social occasions, provided by my host, Dr. Nceba Gqaleni, provided very valuable opportunities to discuss the social implications of the TICIPS research and the very important role provided by the Phytochemistry and Biodiversity Core of TICIPS. The nature of interplant variability, sampling strategy, adequate plant identification and vouchering, essential oils (terpenoids) and the growth of the Traditional Healer industry in South Africa were discussed.

9. Visited the company Plantbio (National Innovation Centre for Plant Biotechnology) in Hayfields Mall, Pietermaritzburg and obtained a copy of their information packet. Making contact with this company was suggested by Dr. Mark Laing.

10. An all-day fieldtrip was undertaken to collect samples of Artemisia afra from the foothills of the Drakensberg Mountains from several locations along the Nottingham Road-Underberg road. The plant collecting was very successful and both herbarium/voucher samples and small bulk samples for phytochemical screening were obtained To protect this South African natural resource, further locality information is only available via myself or one of the participating herbaria (University of the Western Cape & Dunn-Palmer Herbarium, UM-C).

11. Four diverse localities were located and voucher specimens and bulk material of 16 plants of A. afra in these localities were collected.

12. While the above record may suggest that all the activities proceeded as planned, in reality considerable flexing and rescheduling occurred to adjust to changing schedules, agendas and opportunities.

**Relationship building and Research Activity—Cape Town (UWC):**

1. Had considerable conversation with Mr. Frans Weitz regarding his ability to participate in the botanical aspects of the TICIPS research program. Provided Frans with encouragement for him to complete the compilation of his PhD thesis by the end of July. Frans provided me with ‘flimsies’ and ‘blotters’ for my plant press in preparation for fieldwork to collect material of A. afra.

2. Had considerable conversation with Dr. Wilfred Mabusela about our collaborative work on A. afra and the possible need to involve the services of the National Center for Natural Products Research (NCNPR) at the University of Mississippi. Drs Ikhlas Khan and Troy Smillie would oversee this comparative work. The motivation to involve the NCNPR was because they have access to much more sophisticated instrumentation compared with that at UWC.

3. Met with Prof. Quinton Johnson and discussed the growing vision of the South African Herbal Science and Medicine Institute (SAHSMI) at UWC and the struggles associated with the progress of SAHSMI.
4. Mr. Frans Weitz accompanied me on an all-day fieldtrip in the Western Cape. We first visited Mr. Pieter Burger, in the Montagu district (44 Km from the N1 highway, on the R318 towards Montagu) – small-holding called “Tot U Diens”. Mr. Burger is a provider of *A. afra* material for the TICIPS research. Mr. Burger cultivates plants in two small areas on his property. The seed for this cultivation was originally obtained from a local wild population. Mr. Burger indicated that many of the local farmers do not know much about the *A. afra* “Wilde als”. Mr. Burger provides plant material of *A. afra* to the Montagu museum (the museum sells medicinal plant products). A small gratuity was provided to Mr Burger for the plant material he shared with us.

5. *A. afra* plants were collected from both cultivation sites in Mr. Burger’s yard, for voucher specimens and small bulk samples were made for phytochemical screening. GPS locality information was obtained. It was noted that much of the plant material on one of the two sites had already been harvested and supplied to the TICIPS group at the University of the Western Cape.

6. Found another population of *A. afra* plants elsewhere on Mr. Burger’s farmland (apple orchards). This too was sampled for vouchers and phytochemical screening.

7. Visited the Montagu museum to view the medicinal plant garden and the diverse display covering various aspects of medicinal plant trade and use in the Montagu area.

8. Frans Weitz and I then continued our fieldwork in search of other populations of *A. afra* that could be sampled for TICIPS research. Such a large population was discovered in the Robertson district. This population was very large (several/many acres). Several plants were sampled for vouchers and phytochemical screening.

9. In all five localities were located and voucher specimens and bulk samples of 17 plants of *A. afra* were collected.

10. Note that more precise plant collecting locality information is not provided so as to protect the populations sampled. This locality information can be obtained via consultation with me or via application to the University of the Western Cape Herbarium (UWC) or the Dunn-Palmer Herbarium (UMO) at UM-C.

11. Met with Dr. David Fisher (Dept. of Medical Biosciences) who suggested I make contact with Drs Kristi Cammack and William Lamberson (UM-C, ASRC) regarding the importation of small samples of plant material as they had already worked out the USDA APHIS protocol. [Have met with Drs Lamberson and Cammack regarding this and advised about the importance of thorough identification and vouchering).]

12. Visited the Kirstenbosch National Botanical Gardens in Newlands to view their living medicinal plant collection.

13. Paid an unscheduled courtesy visit to Dr. John Manning in the Compton Herbarium, Kirstenbosch National Botanical Gardens. Unfortunately Dr. Manning was away on field-work. Left a note and a copy of the TICIPS poster.

14. Visited the Grand Parade near the Cape Town station to see what medicinal plant material was being traded (on a weekday). Purchased a small sample of *A. afra* which was added to my voucher and phytochemical screening
15. Accommodation during my stay in Cape Town was very adequately provided by iNkosi Lodge (www.inkosilodge.co.za). This bed and breakfast accommodation was relatively close to the UWC campus, with easy access by road, as well as being in a secure neighborhood.

16. Again, while the above record may suggest that all the activities proceeded as planned, in reality considerable flexing and rescheduling occurred to adjust to changing schedules, agendas and opportunities.

During my stop-over in Johannesburg en route back to the USA I visited colleagues at the University of the Witwatersrand (had a delicious social luncheon together) and spoke informally about the TICIPS work. Provided copies of posters. I also paid an unscheduled courtesy visit to Dr. Ben-Erik van Wyk at the University of Johannesburg (previously the Rand Afrikaans University). However, being the Easter season the campus his Department was closed.

Summary of preliminary research:

1. Specimens of *A. afra* collected in KwaZulu-Natal and the Western Cape (Vincent 500 [KZN], Vincent & Weitz 520 & 534 [Western Cape]) have been examined via light microscopy (LM) and scanning electron microscopy (SEM). Collection locality data available via request.

2. Note that the Vincent 520 sample was obtained from the same population of plant material that was harvested for TICIPS research. This material grows on the property of Mr. Pieter Burger in Montagu;

3. Material of *Artemisia annua* (purchased from Ancient Way Acupuncture and Herbs Inc., the same company supplying bulk material for TICIPS), has also been examined;

4. Specimens were examined via light microscopy and scanning electron microscopy with minimal preparation. Chemically fixed samples will be investigated in due course to obtain better quality information. This fixed material is still in the process of being sourced from frozen bulk material at UWC. There have been considerable delays in obtaining this material;

5. The presence of glandular trichomes is readily discerned under 120X magnification;

6. All of the South African samples examined to date possess glandular trichomes (glandular hairs) on: the flowering material (inflorescences); the lower (abaxial) & upper (adaxial) leaf surfaces; the surfaces of young stems (older stems have not been explored yet). There is no evidence (yet) of a glandless biotype of *A. afra*.

7. The material of *A. annua* examined displays the same/very similar distribution of glandular trichomes as that found on the specimens of *A. afra* examined;

8. The appearance of the external micromorphology of the glandular trichomes in *A. afra* matches the published description (Ferreira & Janick, 1995; Int. J. Plant Sci. 156(6):807-815) for *A. annua*. The glandular trichomes are relatively short and have a bi-lobed (dumb-bell) shape. Two images are provided (Figures 1 and 2 in the APPENDIX). Figure 1 is of *A. afra*, Vincent 534, showing glandular trichomes on the upper (adaxial) leaf surface (SEM
view). Figure 2, is of *A. annua* showing glandular trichomes on the upper (adaxial) leaf surface (SEM view);

9. Finer micromorphological/anatomical detail of the glandular trichomes in *A. afra* and *A. annua* have not been compared (this requires tedious fixation, embedding and transmission electron microscopy, which is not the goal of this study). However, based on the overall similarity of the external micromorphology of the glandular trichomes found on these two species, it is fair to presume that the anatomy will be almost identical;

10. Based on preliminary observations, the density (frequency) of the glandular trichomes on the *A. afra* material is at least very similar to that on the *A. annua* material. It could even be more dense;

11. The lower (abaxial) surface of the leaves of *A. afra* is a lot more hairy (tomentose) than similar leaves of *A. annua*. Note that this 'hairiness' is due to the presence of non-glandular T-shaped hairs (trichomes). On both *A. afra* and *A. annua*, these T-shaped hairs are larger than the glandular hairs. Their increased density on *A. afra* results in the relatively short glandular hair-type being partially obscured from view.

12. Eleven samples of *A. afra* covering the range of collection localities and one sample of *A. annua* have been sent to Drs Ikhlas Khan and Troy Smillie at the National Center for Natural Products Research (NCNPR) for phytochemical screening with particular attention on the presence/absence of artemisinin.

13. Some/all the frozen bulk material at UWC is also scheduled for phytochemical screening via Dr. Mabusela's lab.

14. Thorough scrutiny of the collected material indicates that morphometric work involving numerical phenetics, is not really needed as the implied species boundary for *A. afra* appears to be relatively uniform when comparing plant material within KwaZulu-Natal and within the Western Cape and when comparing the material between these two localities. In other words the morphological intraspecific variation appears to be relatively low within and between these Provinces.

15. Work to still be completed:
   a. Phytochemical screening of samples (UWC & NCNPR);
   b. Scanning Electron Microscope (SEM) work on select samples of chemically fixed material of *A. afra*;
   c. Preparation of manuscript possibly in association with Drs Miller and Applequist of the Missouri Botanical Garden. Journal of choice is still to be decided.
   d. Hopefully the lab. work will be completed by the end of 2006.

Balance of budget remaining: Approximately $1,674.

November 9, 2006
Figure 1. *A. afra*, Vincent 534 showing glandular trichomes on the upper (adaxial) leaf surface (SEM view).
Figure 2. *A. annua* showing glandular trichomes on the upper (adaxial) leaf surface (SEM view).
Copy of the Memorandum of Understanding that used in discussions with Traditional Healers in KwaZulu-Natal.

Memorandum of Understanding between collaborators on the TICIPS project

The parties to this agreement affirm:

1. That the conditions of the plant collecting permits granted to Dr. P. Leszek D. Vincent by Ezemvelo KZN Wildlife and CapeNature require that Dr. Vincent provide locality information (latitude & longitude – seconds accuracy), of each plant specimen collected, to the permit-granting authorities;

2. That the latitude and longitude data (seconds accuracy) for the locality of each medicinal plant specimen has implicit intellectual property (IP);

3. That while this precise locality data is requested by the permit granting authorities for their records, it is not made available to the members of the public via any means;

4. That while this precise locality data will be included in the herbarium labeling of each specimen collected via the aforementioned plant collecting permits, that these herbarium specimens are only available to bona fide researchers and are not accessible to members of the general public;

5. That the aforementioned protocol covers the provisions of internationally accepted practices surrounding the collection of plant specimens with associated intellectual property (IP) and/or threatened species status.

That this memorandum of understanding will be upheld by all collaborators on the TICIPS research project (NIH/NCCAM ICRC Cooperative Agreement 1 U19 AT003264-01)

Signed:         (Dr. Leszek Vincent) Date:

Signed:         Date:
South Africa is home to some 200,000 traditional healers and 22,000 allopathic doctors responsible for the care of 44 million people, who derive from many cultures and speak 11 languages. The phytherapies utilized by the traditional healers have been developed over centuries.

AIDS and secondary infections are decimating the populace, overwhelming the dual healthcare system, weakening social and political structures and endangering the prospect of a sustainable, peaceful future for Africa. An estimated 4.69 mil. people in South Africa are infected with HIV. TICIPS will study traditional phytherapies that are being used to treat HIV/AIDS and secondary infections and also suggest novel applications that might be found to be useful.

**Research Project I:** A Randomized, Double-blind, Placebo-Controlled Study of the Safety and Efficacy of *Lessertia frutescens* (L.) Goldblatt & J.C. Manning (syn. *Sutherlandia frutescens* (L.) R. Br.) in HIV-infected South Africans. Co-Leaders: Douglas Wilson (UKZN), Wilsonid1@ukzn.ac.za; Kathleen Goggin (UMKC), Goggink1@umkc.edu.

**Research Project II:** Artemisia afr*.* Artemisia annua and Cervical Cancer. Co-Leaders: Manivasan Moodley (UKZN), Moodleym29@ukzn.ac.za; William Folk (UM-C), FolkW@missouri.edu. and Richard Schlegel (GU) schlgl@aol.com

**Research Project III:** Artemisia afr*.* and TB. Co-Leaders: Muazzam Jacobs (UCT), mjacobs@uct.ac.za; Mark Estes (UTMB), dmesdtes@utmb.edu.

**Research Project IV:** A Phase I Toxicity Trial of the Safety of *Artemisia afr*.* Phytotherapies in Healthy Adults. Co-Leaders: James Syce (UWCC), jayce@uwc.ac.za; Edward Sauter (UM-C), sautere@health.missouri.edu.

**Core 1:** Administration
**Core 2:** Clinical Trials
**Core 3:** Traditional Healers
**Core 4:** Communication
**Core 5:** Phytochemistry and Biodiversity

**NIH/NCCAM** is funding TICIPS for 4 years, with the prospect of further funding. We are interested in working cooperatively with others to expand the scope of TICIPS beyond the present studies.

**Background Information:**
- Approximately 80% of South Africans utilize traditional healers (Luyangas & Sangomas), who employ indigenous phytherapies.
- South Africa has a well documented flora of approximately 3,000 species (~12% of flora) that are used as medicinal plants (colloquially called *muti* - pronounced ‘Motea’).
- The growing interest by the US public towards CAM has prompted the need to ascertain the safety and efficacy of these practices. The NCCAM/NIH wishes to scientifically test CAM practices, including those that impact global health.

**Artemisia afr*.***

**Lessertia frutescens** (family Fabaceae) - among the most widely used *muti* plants in southern African traditional medicine, used in indigenous settings to treat serious microbial infections, especially HIV co-infections; also used to treat wasting conditions and stress-related disorders associated with HIV. Anti-inflammatory properties may favorably influence cytokine networks with possible beneficial effects on quality of life, viral replication and immune function; constituents include pinitol, γ-aminobutyric acid (GABA), and L-canavanine. A Phase I safety trial has been completed by TICIPS and forms the foundation for the Phase III trial in HIV infected adults (Research Project I).

**Other Partners:** Missouri Botanical Garden (MBG), USA; University of Cape Town (UCT), South Africa; University of KwaZulu-Natal (UKZN), South Africa; University of Missouri-Kansas City (UMKC), USA; Georgetown University (GU), USA; University of Texas Medical Branch (UTMB), USA; The South African Medical Research Council (MRC); Representatives of the Traditional Healers community in South Africa.

**Posters by Leszek Vincent (TICIPS, UBC) & William Folk - with assistance from Elizabeth Miller, School of Medicine Office of Medical Research, UBC.**