Superior substrate reported for cultivation of shiitake mushrooms

Research is not an exclusive domain of scientists and this was proved by Vikas Benal, a farmer from Solan district, Himachal Pradesh (HP), who has been cultivating mushrooms for over 23 years. In a recently held symposium, Benal[1] presented his findings on how willow (*Salix* spp.) is a better substrate for cultivation of shiitake mushrooms (*Lentinula edodes*).

In addition to being a culinary delight, shiitake mushroom has many medicinal properties. It has been reported to have antioxidant and anti-cancer properties and is also a good source of folic acid. Shiitake mushroom is a wood-rotting fungus and has been traditionally cultivated on natural wood logs of broadleaf tree species. Though the cultivation of shiitake mushrooms first began in China, it is the Japanese who are the largest producers. In India, the Directorate of Mushroom Research, HP and Indian Institute of Horticulture Research, Bengaluru have developed technology for shiitake mushroom cultivation. The current market price in India for shiitake mushrooms is between Rs 700 and 1000 per kg for fresh and Rs 2000 per kg for the dried form.

Shiitake mushrooms are cultivated on logs or sawdust of non-aromatic broadleaf tree species. Oak (*Quercus* spp.), chinkapin (*Castanopsis* spp.), hornbeam (*Carpinus* spp.), poplar (*Liquidambar* spp.), alder (*Alnus* spp.), beech (*Fagus* spp.), birch (*Betula* spp.), mango (*Mangifera indica*), etc. are considered to be superior quality substrates suitable for shiitake cultivation. Sawdust from trees considered to be of lesser quality is first aged for a suitable period of time before being used. Mycelia of shiitake mushrooms colonize the sapwood which provides them with polysaccharides, but are unable to grow easily into heartwood. Therefore, tree species which have more sapwood are suitable to grow shiitake mushrooms.

As reported by Benal, shiitake mushrooms were cultivated on logs and sawdust of willow. Sawdust proved to be a better substrate as the mushroom crop could be harvested within 45–60 days compared to 90 days or more when cultivated on other substrates[1,2]. An average of 750 g of mushroom per kg of willow sawdust was harvested. However, on willow logs, shiitake mushrooms started fruiting three months earlier than when grown on logs of other tree species with a 30% conversion rate from the start to the harvest of the mushrooms. The technique used is both cost-effective and takes lesser cropping time and needs further studies to determine the science behind it.

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Traditional Knowledge Digital Library: a distinctive approach to protect and promote Indian indigenous medicinal treasure

Interrelationship between nature and Indian society, particularly ethnic/indigenous communities and utilization of bioresources in the healthcare system has a rich legacy. Both codified system of traditional medicine (Ayurveda, Siddha and Amchi) and non-codified medicinal knowledge (folk or indigenous medicine without written texts) have a potential role in the healthcare system and can act as leads for new biologically active molecules or therapy. These types of knowledge have been generated by people during the adaptation and survival process, and accumulated through generations by virtue of tradition[1,2]. Nearly, 75% of the herbal drugs used worldwide were integrated from indigenous medicine and many of the synthetic analogues have been investigated from prototype compounds isolated from plants. Global market for herbal drugs is lucrative, and the trade is expected to reach USD 7 trillion by 2050 (refs 1, 3). In the last few decades the importance of traditional medicinal knowledge has been widely acknowledged, which increases the risk of piracy of such knowledge. Protecting the traditional knowledge (TK) from biopiracy and utilizing them for improvement of health care system are key issues for Government[1,4,5].

Traditional knowledge and Intellectual Property Rights

Intellectual Property (IP) can be explained as 'creations of the human mind, which includes creation or generation of some new and useful things. Intellectual Property Rights (IPRs) are legal rights governing such inventions[6]. Globalization, free trade and the patent regime have created extensive debate regarding the issues related to protection of TK and culture in relation to the Convention on Biological Diversity (CBD) and Trade Related Aspects of Intellectual Property Rights (TRIPS) agreement. The CBD guideline recommends national sovereignty over biological resources, mutually agreed terms and prior informed approval for access to biological resources. TRIPS does not recognize these, but transmits a proprietorship regime and monopolistic intellectual protection[7]. Patent is a tool or process which ensures legal right over a process or product. Three essential criteria to get a patent propounded by TRIPS are: (i) the product or the process should be novel; (ii) it must involve an inventive step (it must not be

evident to a person of common skill) and (iii) it must be useful to society[6,7].

According to the patent law, TK per se refers to already existing documented/undocumented knowledge of indigenous communities, and a patent cannot be granted over it. However, such TK can be used for commercial purposes[8]. Safeguarding TK is a controversial and complicated issue, and discussions are going on internationally regarding safeguarding traditional cultural expressions/folklore and TK, and to address IP access aspects[4,9]. TK is an easily accessible treasure and thus vulnerable to misappropriation. Proper documentation of such knowledge will help prevent biopiracy, boost economy, develop healthcare needs and advance scientific research, promote livelihoods of ethnic/indigenous communities, food security, and preserve cultural, religious, identity and environment.

Biopiracy and Indian traditional knowledge

Biopiracy can be explained as larceny of TK, which also includes violation of a contractual agreement on the access and use of TK and bioprospecting without the permission of the local communities[10]. In the last few decades a number of patents on TK and folk art have been granted, which do not satisfy the basic criteria of novelty. In most of such cases vital information is taken from the traditional communities/ancient scientific literature or with a minor variation thereof without prior informed consent[7].

India, a great land of socio-cultural and ethnic diversity is endowed with vast natural resources and traditional knowledge on medicine. Such knowledge is time-tested, since it has been in practice for centuries[1,5]. Several Indian legislations like the Forest Act, Biological Diversity Act and Forest Rights Act (2006) besides the Indian Patents Act (1970) and its amendments (2002) have recognized the importance and rights related to TK, and have several provisions to protect it[5,8,11]. Despite such laws, biopiracy and unethical bioprospecting of Indian traditional medicine, especially 'codified TK' is a big problem.

Traditional Knowledge Digital Library (TKDL)

The TKDL is a unique proprietary digital database that incorporates knowledge particularly related to medical science from diverse systems like Ayurveda, Unani, Siddha, and Yoga available in the public domain. Information related to healthcare is being documented by sifting and collating the information on traditional knowledge from the available literature existing in local languages. Currently, TKDL is based on 150 books of prior art involving the Indian system of medicine, available at a cost of around USD 1000. The TKDL database is available in different international languages, and thus is accessible to patent examiners in their own mother tongue[12,13]. TKDL contains scanned images of medicinal formulations from ancient original texts, but does not have entire information present in the Indian systems of medicine. TKDL is a dynamic database rather than a comprehensive one, where formulations will be constantly added and continuously updated according to inputs from its users[13–15].

History of TKDL

Between 1990 and 2000, several incidences of biopiracy came to light – most importantly, patents on turmeric (No. 5, 401504 in 1995) and basmati rice (No. 5663484 in 1997) by the United States Patent and Trademark Office (USPTO), and patent on neem by the European Patent Office (EPO; No. 436257 in 1994). These experiences prompted the Government of India (GoI) to formulate a task force which included experts from different sectors to prevent misappropriation of TK at International Patent Offices. TKDL is a collaborative project among the Council of Scientific and Industrial Research (CSIR), Ministry of Science and Technology and Department of Ayurveda, Yoga and Naturopathy, Unani, Siddha and Homoeopathy (AYUSH), Ministry of Health and Family Welfare.

Key features of TKDL[5,12,13]

- Documentation of TK from ancient literature written in regional languages like Sanskrit, Hindi, Arabic, Persian, Urdu, Tamil, etc.
- Documentation of TK related to Ayurveda, Unani, Siddha and Yoga in digitized format.
- Available in English, French, German, Spanish and Japanese. In future, it would be available in 20 foreign languages and all Indian languages.
- Information included from 150 books (75 books on Ayurveda, 10 books on Unani, 50 books on Siddha and 15 books on Yoga) available in the public domain.
- Database contains 1200 formulations (500 Ayurvedic formulations, 500 Unani formulations and 200 Siddha formulations).
- Information on nearly 291 plants which are used as ingredients in these selected formulations, besides ingredients of animal or mineral origin. These formulations are in turn used to treat 186 diseases.
- Access to 2.5 lakh medicinal formulations is available to Patent Offices only under TKDL access agreement. Among them, 82,900 are from Ayurveda, 115,300 from Unani and 12,950 from Siddha.
- Database exists in 34 million A4-size pages.
- Inclusion of information on Yoga is under way. Nearly 900 Yoga postures from 14 ancient yoga books have been transcribed, and will be videographed.

Impact of TKDL on protection of traditional medical knowledge

It has been estimated that a large number of patents relating to Indian medicinal system were being wrongly granted by patent offices around the world. In 2003, nearly 15,000 patents were taken from International Patent Offices related to Indian traditional knowledge, which increased to 35,567 in 2005, and 85,000 in 2008 (refs 12, 14). A survey of 4896 references on 90 medicinal plants mentioned in USPTO database by TKDL task force found that 80% of these references were on seven medicinal plants (Kumari, Mustaka, Tamraparna, Garjara, Atasi, Jambira, Kharbuja) of Indian origin, and 360 out of 762 patents on medicinal plants studied could be characterized as traditional[12,14].

Based on the third party observations submitted by the TKDL team, so far a huge number of patent applications has been either set aside, or withdrawn/cancelled, or declared dead[12]. The innovative TKDL helps India in protecting some 0.250 million medical formulation and at zero cost. Access to TKDL database helps patent examiners find the novelty of patent applications soon after

Table 1. Successful outcome against biopiracy related to traditional medicinal plant using TKDL

Patent Office	Status of patents
European Patent Office	For two patents setting aside of decisions/cancellation of intent to grant patent in 2009; 75 patent applications withdrawn (2009–2013); 31 patents amended/modified due to TKDL prior art evidence (2010–2013); two patents were refused/rejected/cancelled by examiners or application was withdrawn by utilizing TKDL independently without submission of evidences by TKDL (2009–2012).
Canadian Intellectual Property Office	31 patent applications declared 'dead' (2011–2013).
Intellectual Property Australia	One patent application withdrawn (2011); three patents amended/modified due to TKDL prior art evidence (2011–2012).
United States Patent and Trademark Office	Five patents where examiner rejected/cancelled the claims by utilizing TKDL independently without submission of the evidences by TKDL (2011–2012); seven patents amended/modified due to TKDL prior art evidence (2011–2013); four patent claims rejected by the examiner due to TKDL prior art evidence (application is under progress; 2012–2013).
United Kingdom Patent and Trademark Office	Three patent applications terminated (2011–2012).
Controller General of Patents, Designs and Trademarks (CGPDTM) – India	One patent revoked (2012); one patent application was refused/rejected/cancelled by the examiner or application was withdrawn by utilizing TKDL independently, without submission of evidences by TKDL (2012); two patents amended/modified due to TKDL prior art evidence (2013); two patents were refused on the basis of controller's decision.

The patents were claimed by institutions, pharma organizations and individuals of Spain, Italy, The Netherlands, Korea, Denmark, China, Kenya, Germany, USA, India, Russia, Israel, Switzerland, Brazil, Japan, Cyprus, Australia, Canada, Argentina, France, New Zealand, Barbados, Cyprus, Luxembourg, UK, Malaysia, Netherlands Antilles, Malta, Taiwan, Saudi Arabia and Norway. From http://www.tkdl.res.in/tkdl/langdefault/common/Outcome.asp (accessed March 2014).

submission. In the absence of such a database it is difficult to revoke a patent, which is also an expensive and time-consuming process. In general, it takes 5–7 years to oppose a granted patent along with a huge cost ranging between USD 0.2 and 0.6 million. For example, the opposition process to reject the patent of neem (EP436257), Enola beans (US Patent No. 5894079) and Monsanto soybean (EP301749) took 10 years in each of first two cases and 13 years in the third case. Considering this, it is clear that TKDL will not only help prevent the grant of a patent on India's traditional medicine in the early stage, but also reduce the expenditure and time[12,13].

In 2008, inclusion of postures described in Yoga was started after new reports claimed that a large number of false gurus and yoga masters were trying to patent such ancient knowledge. In 2007, nearly 131 yoga-related patents were traced in the US, while a study in February 2004 found 249 patents on Yoga internationally, and 2300 patents, 2315 trademarks at USPTO, and nearly 150 copyrights at USPTO on Yoga were found in May 2005 (refs 5, 14, 16).

In June 2006, GoI agreed to give access of TKDL database to International Patent Offices under Non-disclosure Agreement. Access to TKDL has been provided to EPO (35 member states), German Patent Office, Indian Patent Office, USPTO, Canadian Intellectual Property Office, United Kingdom Patent Office, Intellectual Property Australia and Japan Patent Office[14–16]. Currently, a team from TKDL is involved in the examination of patent applications filed at different patent offices to check incidences of biopiracy or unethical bioprospecting, so that CSIR can submit 'third party observations'. Interestingly, CSIR obtained success in several such cases[14]. Recently, a sharp decline (44%) in the number of patent applications filed relating to Indian medicinal systems, particularly on medicinal plant in EPO has been observed (Table 1)[12].

TKRC – an innovative classification system

TK documentation lacked a proper classification system. Thus an innovative, modern classification system based on the structure of International Patent Classification (IPC) evolved. This system was established for Ayurveda and Unani and has been named as Traditional Knowledge Resource Classification (TKRC). This well-structured classification system for the purpose of systematic arrangement, distribution and retrieval was evolved for nearly 25,000 subgroups against few subgroups present in IPC, related to medicinal plants, minerals, animal resources, effects and diseases, methods of preparations, mode of administration, etc.[5,9,17].

Information is being distributed and arranged under section, class, subclass, group and subgroup according to IPC[10]. TKRC has also encouraged the reform of IPC into 8 sections with approximately 70,000 subdivisions each. Until 2005, only one subgroup (A61K35/78) subsisted for medicinal plants. Based on the observation of India, IPC formed Traditional Knowledge Classification Task Force which includes China, the European Union, India, Japan and the United States, and subsequently, the number of IPC subgroups relating to medicinal plants increased from 1 to 207. These changes initiated a fundamental and far-reaching reform of the international patent system[12].

Till October 2013 about 292,662 transcriptions of traditional medicine formulation in TKDL have been completed, among them 97,203 from Ayurveda, 170,990 from Unani, 22,815 from Siddha and 1654 from Yoga; these are quite significant to realize the objective of the TKDL project. The 'slokas' mentioned in ancient Indian medical texts are read and converted into a structured language using TKRC. TKDL software with its related classification system (TKRC) is

used to translate text in local languages into multiple international languages, which importantly can be viewed as a knowledge-based conversion. Software is also useful to relate the traditional terminology (disease, plant name, etc.) with modern terminology[5,14].

Global importance of TKDL

World Intellectual Property Organization, including the global community has acknowledged TKDL and India's leadership in the area of IPR and TK. Though India presently is not a member of the IPC union, the TKDL database has been chosen for a pilot study by 170 member states of World Intellectual Property Organization. Considering the novelty, importance, efficacy and success of TKDL in preventing the grant of wrong patents, several countries and organizations (like South Africa, African Regional Industrial Property Organization, Mongolia, Nigeria, Thailand, Malaysia) have expressed their eagerness in replicating the TKDL model in their own countries[12,14,15].

Conclusion

TKDL serves the purpose of incorporating information related to traditional knowledge in a common language and in an easy retrieval form, which has enormous benefit in harnessing and developing the traditional knowledge further. TKDL has successfully developed the base to preserve traditional medicinal knowledge of India. TKDL will also increase the scope for active research programmes based on such databases. Furthermore, TKDL will also help India grow economically. It is time to concentrate towards the 'non-codified medicinal knowledge'. Old documents, reports in journals/news bulletin have been found helpful in the process of successful challenge of patents on turmeric, neem and basmati rice. Thus collection and documentation of non-codified knowledge and framework to include these in TKDL will strengthen India's position against biopiracy.

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