TANG [HUMANITAS MEDICINE]

Review

Distribution and phytomedicinal aspects of *Paris polyphylla* Smith from the Eastern Himalayan Region: A review

Angkita Sharma, Pallabi Kalita, Hui Tag*

Pharmacognosy Research Laboratory, Department of Botany, Rajiv Gandhi University, Itanagar-791112, India

ABSTRACT

Comparative studies have established that the North-Eastern (NE) region of India which is a part of the Eastern Himalayan region is affluent in both traditional knowledge based phytomedicine and biodiversity. About 1953 ethno-medicinal plants are detailed from the NE region of India out of which 1400 species are employed both as food and ethnopharmacological resources. Nearly 70% of species diversity has been reported from the two Indian biodiversity hotspots-The Western Ghats and the Eastern Himalayas and these hotspots are protected by tribal communities and their ancient traditional knowledge system. *Paris polyphylla* Smith belongs to the family *Melanthiaceae* and is a traditional medicinal herb which is known to cure some major ailments such as different types of Cancer, Alzheimer's disease, abnormal uterine bleeding, leishmaniasis etc. The major phytoconstituents are dioscin, polyphyllin D, and balanitin 7. Phylogeny of *Paris* was inferred from nuclear ITS and plastid psbA-trnH and trnL-trnF DNA sequence data. Results indicated that *Paris* is monophyletic in all analyses. Rhizoma Paridis, which is the dried rhizome of *Paris polyphylla* is mainly used in Traditional Chinese Medicine and its mode of action is known for only a few cancer cell lines. The current review determines to sketch an extensive picture of the potency, diversity, distribution and efficacy of *Paris polyphylla* from the Eastern Himalayan region and the future validation of its phytotherapeutical and molecular attributes by recognizing the Intellectual Property Rights of the Traditional Knowledge holders.

Keywords Biodiversity hotspots, *Paris polyphylla*, traditional medicinal herb

INTRODUCTION

The Eastern Himalayan illustration

The Himalayas encompass a chain of parallel and converging ranges which makes it the supreme mountain region of the world and among its peaks Mount Everest (8,848 m) is the highest mountain in the world. Due to its enormously active geodynamic conditions even slight alterations in the geo ecolo gy of this area can cause severe changes in the environment that may lead to unsettling consequences (Valdiya, 2001). The Indian Himalayan Region (IHR) which extends up to 250 - 300 km across, stretches over 2.5 km from Jammu & Kashmir in the west to Arunachal Pradesh in the east. It spreads between 21˚57' - 37˚5' N latitudes and 72˚40' - 97˚25' E longitudes. The entire IHR is divided into two agro climatic zones, viz. Zone I comprising of the Western Himalayan region and Zone II comprising of the Eastern Himalayan region. The Eastern Himalayan region also comprises of Central Nepal, Sikkim, China, South-east Tibet, North Bengal and Bhutan and is declared as global Biodiversity hotspot (Myers, 2000). About 70% of species diversity has been reported from the two Indian Biodiversity hotspots-The Western Ghats and the Eastern Himalayas and these hotspots are protected by tribal communities and their ancient Traditional Knowledge (TK) system (Karthi kiyan, 2000).

The North East India (NEI) acts as a refuge to 8 million tribal populations belonging to 150 tribal communities which have diverse and magnificent ethno-cultural heritage. According to the statistics of a few studies, about 1953 ethno-medicinal plants are detailed from the NE region of which 1400 species are employed both as food and ethno-medicinal resources (Albert and Kuldip, 2006; Dutta and Dutta 2005). Arunachal Pradesh, one of the eight states of the NEI covers an area of 83,743 sq.km. An extensive along with an intensive plant collection and survey work was initiated in the state after the reorganization of the Botanical Survey of India in 1955. The state has the richest forest coverage (68%) including precious heritage of flora and fauna belonging to both ecological and ethnobotanical significance and thus falls within the Himalaya Biodiversity hotspots (Hegde, 2002). Such diversity is attributable to the presence of all known climatic zones from Tropical to snow-clad alpine mountains (Kala 2005). 33% of higher plants and 52% of Orchid species of India are found in the forest of Arunachal Himalayas (AH) (Hegde, 2002; Tag et al., 2008). The AH region shelters 26 major tribes and 110 sub-tribes with rich tradition of age-old native knowledge system (Tag and Das, 2004).

There are plenty of literatures based on ethnobotanical scenario from all the states of North East India but only a few dependable literatures on quantitative disease specific ethnopharmacology reliable on precise methodology are available till date (Nima et al., 2009). The mega diverse countries like India and China are reported to have more than 8000 - 5000 years of continuous cultural history and TK. The

*Correspondence: Hui Tag
E-mail: huitag2008rgu@gmail.com
Received Jenuary 1, 2015; **Accepted** August 18, 2015; **Published** August 31, 2015
doi: http://dx.doi.org/10.5667/tang.2015.0001

traditional phytomedicine has the merit over modern allopathic counterpart as the system is verified by ancient traditional and cultural knowledge; other merits are being cheap, lesser side effect, affordability and entirely of a botanical origin (Fabricant and Farnworth, 2001; Patwardhan, 2007). Dose formulation of phytomedicine is suitable based on pragmatic knowledge of TK holders, but need validation in modern laboratory (Jain, 1999; Tag et al., 2008). There are two major approaches to drug discovery; 1. Common approaches which includes Serendipity and Synthetic approach, Chemical Biology approach, and Combinatorial and Genomic approaches which usually takes about 8 - 10 years to isolate the novel unit of the plant. 2. Conversely, current innovative approaches based on Traditional Medicine are- Reverse Pharmacology Approach, Ethnopharmacology Approach, Personalized Approach and Systems Biology Approach which often takes about 4 - 5 years to isolate the novel entity (Tag et al., 2013).

Indian and Chinese custom of agricultural, science, and medicinal practices are yet considered to be time consistent which need firm Intellectual Property Rights (IPR) regulations to protect the rights of the indigenous communities (Tag et al., 2013). Traditional plant-based medicinal products need validation at different stages of pharmacognosy research. This is yet another major focus area where the government should deputize academic and research institutes of the region. Recognizing IPR of TK holders by adhering to the Convention on Biological Diversity (CBD) guidelines have not yet been realized in the NEI (Tag et al., 2013).

Paris polyphylla Smith (English Name: Love apple) belongs to the family *Melanthiaceae* (earlier *Trilliaceae* or *Liliaceae*) and is mostly found in India, China, Bhutan, Laos, Myanmar, Nepal, Sikkim, Thailand and Vietnam. The species are tradtional medicinal herbs and also the major source of raw material used for some medicines, for e.g. 'Yunnan Baiyao', which is well-known for its use as an analgesic and also an anti-coagulant (Long et al., 2002) and also snake-bite therapeutics. Furthermore, it has been used to treat liver cancer in China for several decades (Lee et al., 2005; Shoemaker et al., 2005). Recent diversity study in AH region has confirmed that varieties of *Paris polyphylla* Smith are found in the subtropical and temperate region of Kameng, Subansiri, Kurung Kume, Siang, Lohit, Tirap and Changlang districts (ca 1800 - 3000m) in moist places inside forests. The local communities of the region use the tuber as antidote for snake and insect poison. Based on reports of some anti-cancerous potential of the tubers, the plant has been unsustainably harvested from the wild from the subtropical forest of Himalaya and Eastern Himalaya by the local communities as raw materials for medicinal plant industries which were mostly exported to the countries of Southeast Asia. Such unsustainable collection and harvesting practices has pushed the species on the edge of vulnerability (Tag, 2012). Given the immense medicinal usefulness of the species and impending threat due to overharvesting from the wild, further review is required to understand the ecological adaptation, reproductive viability, feasibility for commercial agrotechnology, medicinal and pharmacological potential of the different varieties and landraces under this significant species found in the Asiatic plate. In a world where indigenous knowledge and science are coming closer ever more, a rational way of dealing with issues of Nature and its values should be presented in a comprehensible way to every person. Thus, the present paper reviews the taxonomy, ecological, medicinal, cultural, biochemical, pharmacological potential and molecular characteristics of *Paris polyphylla* Smith and its varieties found in the Eastern Himalayan region of India.

The genus *Paris*

The temperate genus *Paris* is a perennial herb with slender or thickened rhizome, erect stem and bisexual flowers. Fruit is a berry or a berrylike capsule and is several to many seeded. The genus is precisely divided into 24 species (Ji et al., 2006). *Paris* spp. naturally grow in, montane cloud forests, montane evergreen forests, broadleaved forests, mixed conifer, conifer forests and broadleaved forests and bamboo and scrub thickets (Liang and Soukup, 2000). *Paris* is a plant which is significant in China for its ethnomedicinal value. The classification of *Paris* has long been in dispute and still is unresolved. Based on type of fruit, shape of ovary, seed morphology and shape of rhizome, Takhtajan (1983) divided *Paris* into three major genera: *Paris*, Kinugasa and Daiswa. In recent comprehensive taxonomic revision, subgenus *Paris* (11 species) and subgenus Daiswa (13 species) were recognized which were delimited by axile or partly axile placentation versus parietal placentation, respectively. Subgenus *Paris* was divided into sections Kinugasa (one species), *Paris* (five species) and Axiparis (five species), while subgenus Daiswa was divided into Dunnianae (one species), Marmoratae (two species), Euthyra (eight species), Farge sianae (one species), and Thibeticae (one species) (Ji et al., 2006).

Out of several different species, *Paris polyphylla* belongs to the sub genera Daiswa and section Euthyra and has extreme ethnopharmacological importance. *Paris polyphylla* is further divided into 11 different varieties according to Ji et al. based classification viz. *Paris polyphylla* var. Smith in Rees, Cycl. 26: *Paris* no. 2. 1813, *Paris polyphylla* var. *polyphylla* qi ye yi zhi hua (yuan bian zhong), *Paris polyphylla* var. *yunnanensis* (Franchet) Handel-Mazzetti, Symb. Sin. 7: 1216. 1936, *Paris polyphylla* var. *chinensis* (Franchet) H. Hara, J. Fac. Sci. Univ. Tokyo, Sect. 3, 10: 176. 1969, *Paris polyphylla* var. nana H. Li, Bull. Bot. Res., Harbin 6(1): 123. 1986, *Paris polyphylla* var. *alba* H. Li & R. J. Mitchell in H. Li, Bull. Bot. Res., Harbin 6(1): 123. 1986, *Paris polyphylla* var. *stenophylla* Franchet, No uv. Arch. Mus. Hist. Nat., sér. 2, 10: 97. 1887, *Paris polyphylla* var. minor S. F. Wang, Bull. Bot. Res., Harbin 8(3): 139. 1988, *Paris polyphylla* var. latifolia F. T. Wang & C. Yu Chang in F.T.Wang & Tang, Fl. Reipubl. Popularis Sin. 15: 250. 1978, *Paris polyphylla* var. *pseudothibetica* H. Li, Bull. Bot. Res., Harbin 6(1): 126. 1986, *Paris polyphylla* var. *Kwangtunesis* (R. H. Miao) S. C. Chen & S. Yun Liang, Acta Phytotax. Sin. 33: 490. 1995. Among these varieties, the ones found in India alongwith other neighboring countries are, *Paris polyphylla* Smith, *Paris polyphylla* var. *polyphylla*, *Paris poly phylla* var. *yunnanensis* and *Paris polyphylla* var. *stenophylla* (Ji et al., 2006).

The species *Paris polyphylla*

Paris polyphylla Smith mainly grows in forests, bamboo forests, grassy or rocky slopes, thickets, stream-sides, etc. and grows from 100 - 3500 m. Plants are 10 - 100 cm tall with 1 - 2.5 cm thick rhizome. For the pharmaceutical development of anticancer drugs, natural products have been shown to be brilliant and reliable sources (Mann et al., 2002). *Paris polyphylla* Smith is a monoecious plant where male and female reproductive organs are present in the same flower. The life cycle is concluded in two or more years. It is a slow-germinating plant which takes about seven months to sprout from the seed. *Paris polyphylla* has a wide range of medicinal activities, which includes anticancer, immunoregulatory, and cardiovascular effects (Zhang et al., 2014). The active components present in *Paris polyphylla* Smith are the saponin steroids dioscin, polyphyllin D, and balanitin 7 (Fu et al., 2007). *Paris polyphylla* var. *yunnanensis* grows in moist and humus-

rich soil in woodland conditions where there is full or partial shade. Plants are 30 – 100 cm tall. They grow in coniferous forests, thickets and grassy slopes from 1400 - 3100 m. However, its cultivation is tricky due to long seed dormancy and extremely slow growth from seed. Presently, the wild plant is the lone source of the rhizome. However, due to over collection in recent years the wild plant has become rare and endangered. In order to preserve the natural population and to ensure a steady and renewable source of *Paris polyphylla* var. *yunnanensis* for ethnomedical purposes, thriving cultivation of seedlings and planting is essential (Qi et al., 2013). Freshly matured seeds of *Paris polyphylla* var. *yunnanensis* have undeveloped globular staged embryos. The embryos are quite small relative to size of seed, which otherwise has a large endosperm (Qi et al., 2013). Baskin and Baskin described these plant seeds as the Morphophysiological dormancy (MPD) type with a temperature requirement for breaking dormancy and also embryo growth (Baskin and Baskin, 1998). Post-genome methodologies, like analysis of transcriptomes, metabolomes, proteomes, and bioinformatics have sophisticated our knowledge of seed germination (Mochida and Shinozaki, 2011; Nambara and Nonogaki, 2012). Recently it has been proven that the high-throughput embryos transcriptome sequencing of *Paris polyphylla* seeds is an extremely efficient method for mining genes involved in seed stratification and also dormancy release (Qi et al., 2013). One way of curbing the overexploitation of this medicinal plant is its ex situ conservation (Verma et al., 2011). In relation to TCM, only the rhizomes of two species namely *Paris polyphylla* var. *chinensis* and *Paris polyphylla* var. *Yunnanensis* are recorded officially in Chinese Pharmacopoeia. As the morphological characteristics are quite similar between closely related species, it is thus difficult to differentiate between dry rhizomes of the same genus with the use of Traditional morphological identifycation method, and also time consuming methods like microscopic identification and thin layer chromatography. However, combinations of Near-Infrared Spectroscopy and High Performance liquid Chromatography (HPLC) - based active components along with multivariate analysis have proved to be a powerful method for discerning Paris of different origins and different species (Zhao et al., 2014).

Distribution

The global distribution of *Paris polyphylla* Smith according to Kew Royal Botanical Garden's World Checklist of selected plant families is as follows:

1. *Paris polyphylla* var. Smith

Himalaya to China

36 South Central China (CHC) China North Central (CHN), China Southeast (CHS), China Tibet (CHT), 38 Eastern Asia, Taiwan (TAI) 40 Indian subcontinent, Assam (ASS), Indian subcontinent, East Himalaya (EHM), Indian subcontinent, nepal (NEP) Western Himalaya (WHM) 41 Indo China, Laos (LAO), Indo China, Myanmar (MYA), Indo China, Thailand (THA), Indo China, Vietnam (VIE).

2. *Paris polyphylla* var. *polyphylla*

Himalaya to China

36 South Central China(CHC), North Central (CHN), China Southeast (CHS), China Tibet (CHT), 38 Eastern Asia, Taiwan (TAI) 40 Indian subcontinent, Assam (ASS), Indian subcontinent, East Himalaya (EHM), Indian subcontinent, Nepal (NEP) Western Himalaya (WHM) 41 Indo China, Myanmar (MYA), Indo China, Thailand (THA), Indo China, Vietnam (VIE).

3. *Paris polyphylla* var. *yunnanensis*

SE. Tibet to SC. China

36 South Central China (CHC), China Tibet (CHT) 40 Indian subcontinent, Assam (ASS), Indian subcontinent, East Himalaya (EHM),41 Indo China, Myanmar (MYA).

4. *Paris polyphylla* var. *chinensis*

S. China to Indo-China, Taiwan

36 South Central China(CHC), China Southeast (CHS), 38 Eastern Asia, Taiwan (TAI) 41 Indo China, Laos (LAO), Indo China, Myanmar (MYA), Indo China, Thailand (THA), Indo China, Vietnam (VIE).

5. *Paris polyphylla* var. *nana*

China (S. Sichuan)

36 South Central China(CHC).

6. *Paris polyphylla* var. *alba*

SC. China

36 South Central China (CHC).

7. *Paris polyphylla* var. *stenophylla*

Nepal to C. China

36 South Central China (CHC) North Central (CHN), China Southeast (CHS), China Tibet (CHT), 38 Eastern Asia, Taiwan (TAI) 40 Indian Subcontinent, Assam (ASS), Indian subcontinent, east himalaya (EHM), Indian Subcontinent, Nepal (NEP) Indo China, Myanmar (MYA).

8. *Paris polyphylla* var. *paxiensis*

China (Sichuan)

36 South Central China (CHC).

Paris polyphylla var. minor S. F. Wang, which is listed as a variety of *Paris polyphylla* Smith in the book of Flora of China is otherwise listed as a synonym by the Kew Royal Botanical Garden's World Checklist of selected plant which have instead described *Paris polyphylla* var. paxiensis as one of the variety of the same.

Folk and Traditional Uses

In Nepali folkculture, rhizomes of *Paris polyphylla* have been used as vermifuge and an anti-helminthic (Pande et al., 2007). The powdered roots of the plant are used as ethnopediatrics in case of diarrhoea in Garhwal Himalaya, Uttarakhand, India (Tiwari et al., 2010). *Paris polyphylla* var. *chinensis* and *Paris polyphylla* Smith var. *yunanensis* are the two major and popular folk drugs. *Paris polyphylla* is also used as a folk medicine in Arunachal Pradesh, India where it grows in the altitudes of 9113 - 9891 feet. Its local name is Do-Tala. However, the tribal community of Arunachal Pradesh is still not very conscious about its remarkable medicinal potential (Tag et al., 2012).

The dried rhizome of *Paris polyphylla* Smith var. *yunnane nsis* (Franch.) Hand.-Mazz. or *Paris polyphylla* Smith var. chi nensis (Franch.) Hara is Rhizoma Paridis which belongs to the Liliaceae plant family (Chinese Pharmacopoeia Commission, 2010). It is slightly cold, bitter, mildly toxic, and has the ability to clear heat and remove toxicity, cool the liver, relieve swelling and pain and arrest convulsion. Studies in pharmacology have found that its active ingredients have the anti-tumor effect (Guanglie et al., 2013), haemostatic effect (Fu, 2007), anti-inflammatory effect (Guanglie et al., 2013), antibacterial effect (Guanglie et al., 2013), brain and kidney protection effect (Guanglie et al., 2013), uterine contraction effect (Guanglie et al., 2013) and antioxidant effect (Guanglie et al., 2013), alexipharmic, demulsent, antifebrile, detumescent, haemostatic and the treatment of haemopathy (Cheng et al., 2008). Rhizoma Paridis is the major component of Yun-nan-bai-yao powder, Ji-desen gshe-yao-pain tablets and Gongxuening capsules which are well known organized Chinese medicines.

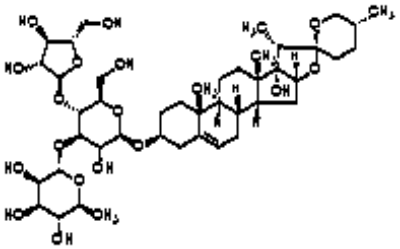

Fig. 1. Structure of Polyphyllin D (Lee et al., 2005).

It has frequently played a significant role in clinics for treating fractures and immunity adjustment and aprotitis, analgesia, tumours, and bleeding (Zang et al., 2010). Also it plays a significant role in antifertility, malady, and spermicidal enhancement and sedative (Cheng et al., 2008) Gongxuening capsules have been extensively used for more than a decade and are commercially accessible in most cities in China (Pande et al., 2007). Typically, this patent medicine is used to cure female patients who suffer from menorrhagia, metrorrhagia, metrostaxis, functional uterine bleeding and chronic pelvic inflammatory 328 X.X. diseases (Guanglie et al., 2013).

HERBARIUM COLLECTION

The herbarium catalogue from the Royal Botanical Garden Edinburgh exhibited herbarium for some of the varieties of *Paris polyphylla* Smith which are as follows:

1. *Paris polyphylla* Sm. var. *polyphylla*; Collector/Expedition: Cavalerie, Pierre Julien. Collection Number: 533; Collection Date: 24 September, 1902; Filling region: Inner China, Korea and Taiwan; Country of Origin: China; holotype of Paris debeauxii H. Lev. Barcode: E00346110.
2. *Paris polyphylla* Sm. var. *stenophylla* Franch; Collector/Expedition: Maire, E.E. Collection Number: S.N; Collection Date: May, 2009; Filling region: Inner China, Korea and Taiwan; Country of Origin: China; holotype of Paris hamifer H. Lev. Barcode: E00346106.
3. *Paris polyphylla* Sm. var. *yunnanensis* (Franch.) Hand.-Mazz. Collector/Expedition: Bodinier, Emile M. Collectio n Number: S.N; Collection Date: May, 2009; Filling region: Inner China, Korea and Taiwan; Country of Origin: China; holotype of Paris christii H. Lev. Barcode: E00346 104.
4. *Paris polyphylla* Sm. var. *yunnanensis* (Franch.) Hand.-Mazz. Collector/Expedition: Cavalerie, Pierre Julien. Collection Number: 1310; Collection Date: 13 June 1902; Filling region: Inner China, Korea and Taiwan; Country of Origin: China; holotype of Paris cavalerie H. Lev. Barcode: E00318494.
5. *Paris polyphylla* Sm. var. *yunnanensis* (Franch.) Hand.-Mazz. Collector/Expedition: Cavalerie, Pierre Julien. Collection Number: 729; Collection Date: 23 November 1902; Filling region: Inner China, Korea and Taiwan; Country of Origin: China; holotype of Paris gigas H. Lev & Vaniot. Barcode: E00346105.
6. *Paris polyphylla* Sm. var. *yunnanensis* (Franch.) Hand.-Mazz. Collector/Expedition: Bodinier, Emile M. Collection Number: 1635; Collection Date: 17 July 1898; Filling region: Inner China, Korea and Taiwan; Country of Origin: China; holotype of Paris mercieri H. Lev & Vaniot. Barcode: E00346107.
7. *Paris polyphylla* Sm. var. *yunnanensis* (Franch.) Hand.-Mazz. Collector/Expedition: Maire, E.E. Collection Number: S.N; Collection Date: 17 July 1898; Filling region: Inner China, Korea and Taiwan; Country of Origin: China; holotype of Paris atrata H. Lev & Vaniot. Barcode: E00346103.
8. *Paris polyphylla* Sm. var. *yunnanensis* (Franch.) Hand.-Mazz. Collector/Expedition: Cavalerie, Pierre Julien. Collection Number: 2023; Collection Date: 05 June 1907; Filling region: Inner China, Korea and Taiwan; Country of Origin: China; holotype of Paris pinfaensis H. Lev & Va niot. Barcode: E00318495.
9. *Paris polyphylla* Sm. var. *yunnanensis* (Franch.) Hand.-Mazz. Collector/Expedition: Cavalerie, Pierre Julien. Coll ection Number: 3023; Collection Date: 25 June 1907; Fil ling region: Inner China, Korea and Taiwan; Country of O rigin: China; holotype of Paris aprica H. Lev & Vaniot. B arcode: E00318500.
10. *Paris polyphylla* Sm. var. *yunnanensis* (Franch.) Hand.-Mazz. forma velutina H. Li & Noltie. Collector/Expeditio n: Kunming, Edinburgh, Gothenburg Expedition (1993). Collection Number: 304; Collection Date: 26 May 1993; Filling region: Inner China, Korea and Taiwan; Country of Origin: China; holotype of *Paris polyphylla* Sm. var. y unnanensis (Franch.) Hand.-Mazz. forma velutina H. Li & Noltie. Barcode: E00346102.

PHYTOCHEMISTRY

The chemical constituents of *Paris polyphylla* Sm. are mainly Paris saponins which account for more than 80% of the total compounds of which diosgenin plays the major part. Along with Paris saponin I (diosgenin 3-O-α-L-rha-(1-2)-[α-L-arab-(1-4)]-β-D-glu), Paris saponin II (diosgenin3-O-α-rha-(1-4)-α-L-rha-(1-4)-[α-L-rha-(1-2)]-β-Dglu), Paris saponin III, diosgenin and C22-methoxy-protodioscin,C22-hydroxy-protodioscin, C22-methoxy-protopolyphyllin I, C22-hydroxy-protopolyphyllin I, C22-methoxy- protopolyphyllin II, (Wu et al., 2004), polyphyllin VI, and polyphyllin VII are other important compounds found in this plant.

In 2005, Devkota isolated 6(six) compounds from *Paris polyphylla* which he collected from Parbat district Nepal (Pande et al., 2007).

These compounds are:

a) Saponin-1 (Diosgenin-3-O[α-Lrhamnopyanosyl (1Rha-2Glu)-α-Lrhamnopyranosyl(1Ara-4Glu)]-β-DGlucopyranoside) which has a steroidal skeleton (Fig. 4).

b) Polyphyllin C (Diosgenin-3-O[α-L rhamnopyanosyl(1-3)- α -D-glucopyranoside) which has a steroidal skeleton

c) Polyphyllin D (Diosgenin-3-O[α-Lrhamnopyanosyl (1Rha-2Glu)- α –Larabinofuranosyl(1Ara-4Glu)]-β-DGlucopyrano-side) which has a steroidal skeleton (Fig. 1).

d) Przewalskinone B (1,5-Dihydroxy-7-methoxy-3-methylanthr aquinone) which has a anthraquinone skeleton

e) Stigmasterol which is a steroid, and

f) Stigmasterol-3-O- β -D-glucoside.

12 steroidal saponins from *Paris* were isolated by Zhou Zhun of Kunming Institute of Botany and grouped the 12 saponins into three groups-diosgenin, pennogenin and 24-α-hydroxy pennogenin saponins (Jun, 1989).

New saponins-polyphyllin A-H has been isolated from the

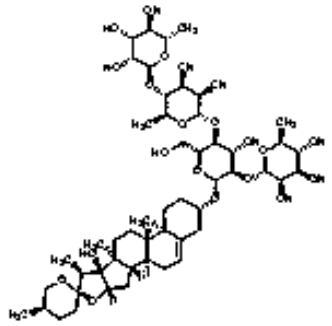

Fig. 2. Pennogenin Tetraglycoside (Wang et al., 2012).

rhizome of *Paris polyphylla* of which first six are spirostanol steroidal saponins and remaining two are furastanol steroidal sa ponins (Rastogi and Mehrotra, 1993). Pennogenin Tetraglyco side (Fig. 2) is one of the spirostanol-type steroidal saponins which induce contractile activity in the myometrium, extracted from *Paris polyphylla* Sm. var. *yunnanensis* (Wang et al., 2012).

Huang et al in 2007 extracted a novel steroidal saponin together with the 12 known compounds from *Paris polyphylla* var. *chinensis* (Yun et al., 2007). The novel compound was attained as an amorphous solid and spectral data including two dimensional NMR showed the structure as 3b,21-dihydroxy pregnane-5-en-20S-(22,16)-lactone-1-O-a-L-rhamnopyranosyl (1® 2)-[b-D-xylopyranosyl(1® 3)]-b-D-glucopyranoside. The 12 known compounds are identified steroids and their structures were recognized by 13C NMR spectrum (in pyridine-d5) as

1. diosgenin (Fig. 5).,
2. pennogenin,
3. diosgenin-3-*O*-a-L-rhamnopyranosyl(1→2)-b-D-glucopyranoside,
4. pennogenin-3-*O*-a-L-rhamnopyranosyl(1→2)-b-D-glucopyranoside,
5. diosgenin-3-*O*-a-L-rhamnopyranosyl(1→2)[a-L-arabinofuranosyl(1→4)]-b-D-glucopyranoside,
6. pennogenin-3-*O*-a-L-rhamnopyranosyl(1→2)[a-L-arabinofuranosyl(1→4)]-b D-glucopyranoside,
7. diosgenin-3*O*-a-L-rhamnopyranosyl(1→2)-[b-D-glucopyranoside(1→3)]-b D-glucopyranoside,
8. diosgenin-3-*O*-a-L-rhamnopyranosyl(1→4)-a-L-rhamnopyranosyl(1→4)[a-L-rhamnopyranosyl(1→2)]-b-D-glucopyranos ide,
9. pennogenin-3-*O*-a-L-rhamnopyranosyl(1→4)-a-L-rhamnopyranosyl(1→4)[a-L-rhamnopyranosyl(1→2)]-b-D-glucopyranos ide,
10. 3-*O*-a-L-arabinofuranosyl(1→4)[a-L-rhamnopyranosyl(1→2)]-b-D-glucopyranoside-b-D-chacotriosyl-26-*O*-b-D-gluc opyranoside,
11. 2b,3b,14a,20b,22a,25b hexahydroxycholest-7-en-6-one, and
12. 2b,3b,14a,20b,24b,25b hexahydroxycholest- 7-en-6-one.

Recently, a rapid and specific LC-MS-MS technique was developed for the analysis of polyphyllin I (Fig. 6)., polyphyllin II, polyphyllin VI and polyphyllin VII in the plasma of beagle dog after oral administration of Rhizoma Paridis extracts (RPE), which had recovery, precision , high sensitivity, accuracy and reproducibility (Yina et al., 2013).

In a study conducted in 2011, the content of mineral elements (Ca, Cr, Cu, Fe, K, Mg, Mn, Na, and Zn) in *Paris polyphylla* var. *yunnanensis* from Yunnan, Guihou, and Guangxi of China was assessed. The contents were found in the order of Ca > K > Mg > Fe > Na > Cu > Mn > Zn > Cr. This might have a relationship with the physiological responses of the species. The results proposed that *Paris polyphylla* var. *yunnanensis* is a worthy source of Ca, K, Mg, Fe, Na and Cu (Zhang et al., 2011).

PHARMACOLOGICAL INDEX

Cervical cancer

Cervical cancer is considered to be the third most common type of cancer in women and was responsible for 275,000 deaths in 2008 (Ferlay et al., 2008). Despite of techniques such as neoadjuvant chemotherapy, platinum-based anticancer agents, represented by cisplatin, which have therapeutic properties, the survival rate has not improved in case of cervical cancer. Toxicities, including leukopenia, myelosuppression, neuro-toxicity, ototoxicity, and nephrotoxicity (Alderden et al., 2006; Boulikas et al., 2007) may restrict their long-term use. In a recent study, the mechanism involved in the cytotoxic effects of a particular steroidal saponins from Chonglou (Rhizoma Paridis Chonglou), namely, Paris saponin VII (PS VII) (Fig. 3), alongwith its antitumor properties on Hela cell lines was tested. The results showed that PS VII inhibited the growth of the cell effectively. PSVII also upregulated cleaved caspase-3, cleaved caspase-9, and Bax expression in Hela cells. These results indicated that PS VII may have potentials in the cure of cervical cancer (Zhang et al., 2014).

Human gastric cancer

Cell apoptosis is directly related to the incidence, development and metastasis of tumors (Aneja et al., 2008; Kim et al., 2008, Hung et al., 2008). The methods of apoptosis in tumor cells is a significant field of study for treatment of tumor and molecular cancer biology (Sutter et al., 2006).The progression of cells in the course of the cell cycle is powered by the sequential activation and inactivation of a family of serine-threonine kinases called the cyclin-dependent kinases (CDKs). Particularly, CDK1 controls progression from the S phase through G2 and into the M phase. Likewise, progression from the G1 to S phase is managed sequentially by CDK4/6 and CDK2. CDK activity is synchronized by binding to cyclin partners and the act of endogenous inhibitory peptides (Morgan et al., 1995; Norbury et al., 1992). The recognition of strong and selective cyclin dependent kinase inhibitors is the main concern for anti-cancer drug discovery.

PCD or *Paris chinensis* dioscin uses its inhibitory effects on SGC-7901 cells by stirring up G2/M cell cycle arrest and Ca^{2+} - cytochrome C- apoptosis. PCD may be a possible candidate as a unique therapeutic agent that originates from a natural source, and the initiation of apoptosis by PCD in other cancer cell lines is the topic of on-going analysis (Gao et al.,

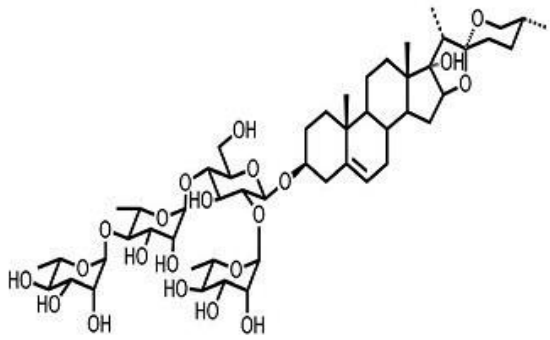

Fig. 3. Structure of Paris Saponin VII (Zhang et al., 2014).

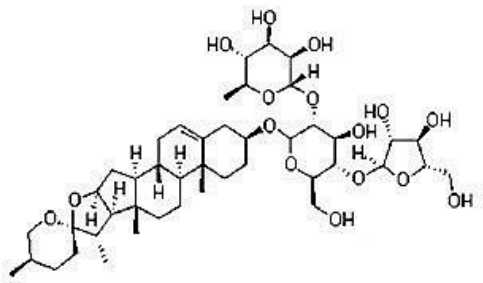

Fig. 4. Paris Saponin I (Jiang et al., 2014).

2011).

Polyphyllin D is a major steroidal component derived from Polyphyllin D and dioscin evade drug resistance and generate apoptosis in liver cancer HepG2, R-HepG2, cells (Cheung et al., 2005; Deng et al., 1999; Gao et al., 2011; Li et al., 2001).

Zhu et al., 2011 showed that B-ecdysterone and three other compounds having the same steroidal saponin moiety were isolated from *Paris polyphylla* var. *yunnanensis* and the three pennogenin steroidal saponins inhibited HepG2 cancer cell proliferation and also induced cancer cell apoptosis.

Lung cancer

Lung cancer is reported to have become one of the chief causes of cancer related mortality all over the world of which non small cell lung cancer (NSCLC) represents 80% of lung can cers (Sordella et al., 2004; Workman 2004). Epidermal growth factor (EGFR) is a major therapeutic target in NSCLC (Kobayashi N et al., 2012). Patients having somatic mutations of the kinase domain of EGFR usually react to tyrosine kinase inhibitor (TKI) therapy, though often show progressive disease following 6 8 months of therapy (Jackman et al., 2006; Riely et al., 2006). Radiotherapy is tremendously important for patients with NSCLC who cannot go for surgery and patients who have experienced chemotherapy or TKI therapy failure. Nevertheless, NSCLC cells are normally less sensitive to radiotherapy when compared to SCLC cells, which results in radiotherapy failure (Riely, 2002). Even then radiotherapy leads to serious side effects, which includes irradiation pneumonitis and also a repressed hemopoietic system. In recent decades, focus has been made on identifying biologically vigorous cancer therapeutic agents obtained from natural resources (Grabley and Thiericke, 1999). Paris saponins (PSI), mainly from *Paris polyphylla* Smith var. *yunnanensis* (Franch) Hand Mazz exhibit ted effective radiosensitivity against gefitinib resistant PC 9 ZD cells in vitro which was associated with the cell cycle arrest at the G2/M phase and apoptosis by enhanced caspase 3, Bax and P21waf1/cip1 and reduced Bcl2 production (Jiang et al., 2014).

Esophageal cancer

The results of *Paris polyphylla* Smith (PPSE) on human esophageal cancer ECA109 cells and also the signaling pathways involved in PPSE-induced apoptosis were not properly known. Gap junction (GJ) links the cytoplasm of neighboring cells. They settle the direct transfer of low-molecular-weight metabolites and ions, which includes second messenger like inositol triphosphate, Ca^{2+} and cyclic AMP, between adjacent cells (Oyamada et al., 2005). GJ channels are formed by two hemi channels (connexons), further each connexon is composed of six distinct trans-membrane proteins called connexins (Martin et al., 2004). Certain connexins have tumor suppressing effect, including connexin32, connexin43, and connexin26 (Decrock et al., 2009; Fujimoto et al., 2005; Tanaka et al., 2004). Connexin 26 is one of the very commonly investigated Connexin proteins which shows growth inhibition and induction of apoptosis (Tanaka et al., 2004). In a recent study, connexin26 enhancement by PPSE and also inhibition of the augmentation and propagation partly by reduction of Bcl-2 and increased Bad in ECA109 cells has been shown (Li et al., 2012).

Human breast cancer

It was established in a study conducted a few years back that treatment of MCF-7 and MDA-MB-231 cells with polyphyllin D results in the reticence of viability and initiation of apoptosis, with an IC50 of 5 microM and 2.5 microM, respectively, shown after 48h of incubations. It was evidenced by the formation of a hypodiploid peak in the cell cycle analysis, phosphatidyl-serine externalization, incidence of DNA fragmentation and a late loss of membrane integrity. The mechanism of action suggests that polyphyllin D dissolves the mitochondrial membrane potential, encourages a down-regulation of anti-apoptotic Bcl-2 expression and an up-regulation of pro-apoptotic Bax expression, and triggers caspase-9. Such results imply that polyphyllin D brings out apoptosis through mitochondria dysfunction. In vivo intravenous injection studies revealed that daily administration of polyphyllin D (2.73 mg/kg body weight) through ten days in nude mice bearing MCF-7 cells efficiently lessened tumor growth for 50% in conditions of tumor weight and size, and shows no significant toxicity in liver and heart to the host. These findings impart original insights that polyphyllin D could operate as a candidate in breast cancer treatment (Lee et al., 2005).

Alzheimer's disease

Loss of synapses and Neuritic atrophy cause the pathogenesis of Alzheimer's disease (AD) and are situated upstream of neuronal death in the Ab cascade (Dickson and Vickers, 2001; Terry et al., 1991). The dysfunction of synapses and neurites is the exact cause of the memory deficit in AD. As neurons having atrophic neurites may continue to be viable and also have the ability to be remodeled, the vital event for the attainment of revival of brain function after injury is the renovation of neuronal networks, including synaptic reformation (Tohda et al., 2005) and neurite regeneration. Diosgenin, a steroidal saponin has several biological effects. A diosgenin derivative, caprospinol (diosgenin 3-caproate) has been reported to reduce amyloid deposits and also to improve memory dysfunction in Ab1-42-infused AD model rats (Lecanu

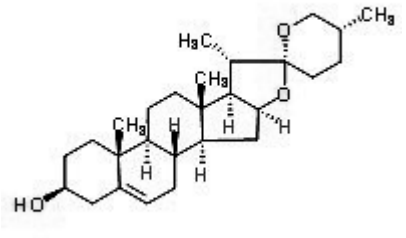

Fig. 5. Diosgenin (Raju and Rao 2012).

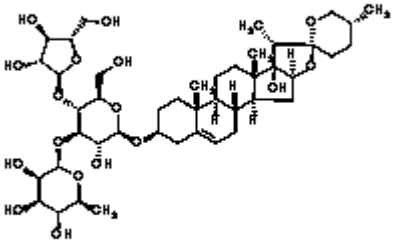

Fig. 6. Polyphyllin I (Yue et al., 2013).

et al., 2010). A recent study showed that diosgenin administration increased the object detection memory deficit in 5XFAD mice. Diosgenin administration reduced several signs of neuronal degeneration, which includes presynaptic disintegration combined with amyloid plaques in the cortex, axonal degeneration associated with amyloid plaquees in the cortex, hippocampus and cortex and PHF-tau express ion associated with and distal to amyloid plaques in the cortex and hippocampus. In addition, amyloid plaques were decreased by diosgenin treatment. The amyloid plaque reducing effect of diosgenin results in the hyperphosphorylation and the safety of axonal degeneration of tau. Thus, diosgenin may add to axonal extension in a direct pathway, and also through the amyloid plaque-lowering pathway (Tohda et al., 2012).

Abnormal uterine bleeding

Abnormal uterine bleeding (AUB) is one of the major fields of concern for gyenocologists worldwide. Approximately one-third of all gynecological consultations are carried out for AUB which rises to 70% in women premenopause and postmenopause (Oehler et al., 2003). This consists of both dysfunctional uterine bleeding, which can be anovulatory or ovulatory, plus bleeding due to structural causes, including polyps, fibroids, pregnancy complications and endometrial carcinoma (Ely et al., 2006). Additionally, AUB can also result from contraception (Schrager, 2002). Because of the high morbidity, therapy of AUB is much important for women's healthcare. In search for definitive cure, treatments like hysterectomy and drugs like combinations of estrogen and progestin, progestins, plasminogen inhibitors, such as tranexamic acid and prostaglandin synthetase inhibitors are commonly used. Total steroidal saponins of *Paris polyphylla* Sm. var. *yunnanensis* (TSSP) have been constantly used in China for the treatment of AUB. Bioassay-guided separation technique showed that total spirostanol saponins exhibited contractile activity in myometrium and Penno genin-3-O-a-L-arabinofuranosyl (1→4)[a-L-rhamnopyranosyl (1→2)]-b-D-glucopyranoside (PARG) was proven as the active ingredient of TSSP (Guo et al., 2008)

Antioxidant property

Reactive oxygen species (ROS), like hydroxyl radicals (OH•), superoxide anion (O_2•-), hydrogen peroxide (H_2O_2), and singlet oxygen ($^1O_2$) play a chief role in the advancement of oxidative stress which leads to many illnesses including diabetes, cardio vascular diseases, degenerative diseases, inflammation, anemia, cancer and ischemia (Cai et al., 2004). Synthetic antioxidant agents come with drawbacks such as lack of availability, high cost, and side effects. Plant based antioxidant compounds due to their inability to cause side effects (Cai et al., 2004; Drag land et al., 2003) play a defensive part by averting the production of free radicals and therefore are enormously valuable to lessen the diseases initiated by oxidative stress (Akinmoladun et al., 2010; Ö zen et al., 2010). In addition to anti-oxidant property the phenols and flavonoids act as brilliant anti-inflammatory agents (Talhouk et al., 2007; Zhang et al., 2011) against pro-inflammatory molecules such as TNF-alpha, NO etc. These molecules also causes tissue damage and cell death as NO can retort with the free radicals such as superoxide to generate peroxynitrite, that leads to irreversible injury to cell membranes (Lee et al., 2005; Wang et al., 2004). Many plants belonging to TCM along with *Paris polyphylla* is reported to possess such effects (Ravipati et al., 2012).

Anti-fungal activity

The antifungal test of compounds from *Paris polyphylla* var. *yunnanensis* gave the results which showed that certain compounds isolated had antifungal activities against Saccharomyces cerevisiae hansen and Candida albicans.

Anti-bacterial action

The roots of *Paris polyphylla* have shown anti bacterial action against *Bacillus dysentery*, B. *paratyphi*, B. *typhi*, *Staphylaco ccus aureas*, *Escherichia coli*, *Haemolytic streptococci*, *Menin gococci* etc.

Spermicidal action

The plant extract of *Paris polyphylla* exhibited effective spermicidal activity against human and rat sperms. The vaginal treatment of the plant's extract (100 mg/animal) barred pregnancy upto 60% of the rabbits tested (Pande et al., 2007).

Tyrosinase inhibitory activity

Tyrosinase (EC 1.14.18.1) is a multifunctional enzyme containing copper which is widely dispersed in plants and animals. It performs like a catalyst in the oxidation of monophenols, o-di phenols, and o-quinones which is also a vital enzyme in melanin biosynthesis in plants and animals. Thus, tyrosinase inhibitors can be clinically functional for the cure of some dermatological disorders which are associated with melanin hyper-pigmentation. These are also useful in cosmetics for de-pigmentation after sunburn and whitening. Furthermore, tyrosinase is involved in the molting procedure of insects and adhesion of marine organisms (Shino et al., 2001).

The extracts, compounds and fractions of *Paris polyphylla* in a study showed to possess inhibitory activity against the enzyme tyrosinase (Devkota et al., 2007).

Anti-leishmanial activity

Leishmaniasis is a parasitic disease which is caused by the attack of intracellular parasite known as Leishmania in the reticulo-endolethial system of the host. They are transmitted from host to host by the bite of a vector sand fly (Ram et al., 1996). It is considered to be one of the most dreadful diseases and is a major health problem of tropical, subtropical, and Mediterranean regions (Berman, 1998). The disease is classified on the basis of visceral (kalaazar), symptomatology as cutaneous, mucosal or micro cutaneous and diffused cutaneous. No safe and efficient vaccine is yet available for leishmaniasis and thus chemotherapy is the lone means of controlling the disease.

The extracts, compounds and fractions of *Paris polyphylla* in a study showed to possess inhibitory activity against the enzyme tyrosinase and also anti-leishmanial activities (Devkota et al., 2007).

Immunostimulating property

The presence of glucoside moieties of diosgenyl saponins is needed for the commencement of immunological reactions, particularly during the period of oxygen expenditure such as in the process including microbial activity and inflammation although diosgenin could only stimulate the macrophages phagocyte sis including elimination of foreign or denatured matter (Zhang et al., 2007).

Anti-pyretic activity

Paris polyphylla also finds its utility in ailments such as fever and headache (Uprety et al., 2010).

Others

Apart from such major uses, the rhizome of *Paris polyphylla* contains sugars (7.9%) and two glycosides viz α-paridin (m.p. 244 - 460) and α-paristypnin (m.p. 147 - 490) which creates a tingling sensation on the tongue (Pande et al., 2007). α-paristypnin has a depressant action on myocardium, carotid pressure and respiratory movements. It produces vasoconstriction in kidney, but vasodilation in the limbs and spleen and stimulates the intestines (Pande et al., 2007).

The haemostatic activity of the rhizome of *Paris polyphylla* is due to saponins of pennogenin. Studies have suggested that *Paris polyphylla* also has antihelminthic activity (Watanabe et al., 2005). *Paris polyphylla* is also used in treatment of trauma and anti-parotitis.

Side-effects

Paris polyphylla var. *yunnanensis* is normally considered safe. Nevertheless pregnant or lactating women should abstain from using the herb except otherwise affirmed by their General Practitioner.

MODE OF ACTION

Proteomics made a major contribution to the mechanistic investigation of TCM mixtures or decoctions. It was reported in 2008 that the total saponin of the rhizomes of *Paris polyphylla* Smith var. *chinensis* (Franch) Hara can induce apoptosis in HepG2 cells by the process of up-regulation of hnRNPK, dUTPase, GMPsynthase and down- regulation of nucleoside diphosphate kinase A, DNase gamma, and centrin-2-as established by proteomics (Cheng et al., 2008). Paris saponins also had significant inhibitory effect on in vitro U14 Cervical cell line, where in vivo, it could inhibit a significant number of tumor cells in tumor-bearing mice. Here, serum IFN-γ level increased while IL-4 level significantly reduced thereby activating the immune response (GuangLie et al., 2013).

In natural environment, the internal tissues of most TCM plants including *Paris polyphylla* are colonized by various differrent microorganisms which are termed endophytes. These endophytes produce secondary metabolites which provide vital ecological benefits to the host plant. Thus, such endophyte derived metabolites could be the cause of bioactivity and associated favorable health claims of the TCM host plants. A study to screen such potential of endophytes using polykletide synthase (PKS) and non-ribosomal peptide synthetase (NRPS) genes as proxy for endophytic production of bioactive compounds was made and the results were positive for some TCM host plants. Thus, plants that host endophytes with great potential to produce polykletide and non-ribosomal peptide-based bioactive substances should be focused. (Miller et al., 2012).

MOLECULAR EVALUATION

The chromosomal number of *Paris polyphylla* Sm. var. *yunnanensis* is 2n = 10 (Miyamoto et al., 1992). Phylogeny of Paris was inferred from nuclear ITS and plastid psbA-trnH and trnL-trnF DNA sequence data (Ji et al., 2006). Results indicated Paris is monophyletic in all analyses. In ITS analysis, *Paris polyphylla* Sm. var. *yunnanensis* was sister to *P. luquanensis*. *Paris polyphylla* chloroplast matK gene for maturase, complete cds is present in the sequence database. DNA-based molecular markers are adaptable tools and have been used in various fields such as taxonomy, embryology, physiology, genetic engineering, etc. Since their development, they have been constantly customized to improve their exploitations and to be of added importance for the analyses of the genome and genetic diversity (Gonçalves et al., 2009; Oliveira et al., 2010 Vieira et al., 2007). Molecular markers allow us to make approximates of genetic diversity directly at the DNA level, plummeting the interference of environmental factors (Cabral et al., 2011). Further, many markers exist for each genome, they are free of pleiotropic effects and there are high levels of polymorphism (Leal et al., 2010). Recent decades, SSRs (simple sequence repeats), also known as microsatellites, have become a very popular source of genetic marker because of their multi-allelic nature, high reproducibility, high abundance, co-dominant inheritance, and wide genome coverage (Cabral et al., 2011; Demir et al., 2010; Oosterhout et al., 2004; Sharma et al., 2007). The high information content in SSR loci along with their codominant expression make SSRs suitable for population genetics, genomic mapping, marker-assisted selection, conservation bio logy and other studies (Ellegren, 2004; Leal et al., 2010; Roa et al., 2000; Schlötterer, 2004). Even though the morphology of *Paris polyphylla* has been extensively studied, information about its population genetics and molecular phylogeny is very scarce. In a recent study, the isolation and characterization of 12 polymorphic microsatellite loci *from Paris polyphylla* var. *chinensis* by using a biotin-capture method (Bloor et al., 2001), together with the analysis of the genetic diversity of *Paris polyphylla* var. *chinensis* using SSR molecular markers have been studied.

CONCLUSION

Paris polyphylla Smith is a rare and one of the most significant medicinal plants found in the North Eastern part of India of the Eastern Himalayan Region. Its effective ingredients are found to actively cure many small and major ailments including cancer and Alzheimer's disease. It has found its use in many tradetional and folk medicine in China, Nepal and North East India. The molecular profiling of this plant however is in the nascent state. Most of the pharmacognostic studies have been evaluated for *Paris polyphylla* from the Eastern Himalayan region but some are yet to be done. Current literature in phytomedicine discovered that around 80% of population in developing countries like India relies on traditional herbal medicine. However, competence in traditional phytomedicinal research area should be raised, along with the quality control and consistency of such products. Indian and Chinese custom of agricultural, science, and medicinal practices which are yet considered to be time consistent actually need firm IPR regulations to protect the rights of the indigenous community. Hence, extensive further studies in this marvel plant from the Eastern Himalayan region should be of the key focus for the

scientific community related to phytotherapy and biodiversity conservation.

ACKNOWLEDGEMENTS

The authors are thankful to University Grant Commission New Delhi for supporting funding under Centre with Potential for Excellence in Biodiversity (CPEB II) project. The last author is thankful to UNDP India for funding support provided during field and literature work. Administrative and logistic support extended by authorities of Rajiv Gandhi University Arunachal Pradesh is duly acknowledged. The authors have no conflicting financial interests.

CONFLICT OF INTEREST

The authors have no conflicting financial interests.

REFERENCES

Akinmoladun AC, Obuotor EM, Farombi EO. Evaluation of antioxidant and free radical scavenging capacities of some Nigerian indigenous medicinal plants. J Med Food. 2010;13:444-451.

Albert LS and Kuldip G. Traditional use of medicinal plants by the Jaintia tribes in North Cachar Hills district of Assam, northeast India. J Ethnobiol Ethnomed. 2006;2:33.

Alderden RA, Hall M, Hambley TW. The discovery and deve lopment of cisplatin. J Chem Educ. 2006;83:728-734.

Aneja R, Liu M, Yates C, Gao J, Dong X, Zhou B, Vangapandu SN, Zhou J, Joshi HC. Multidrug resistance-associated protein-overexpressing teniposide-resistant human lymphomas undergo apoptosis by a tubulin-binding agent. Cancer Res. 2008;68:1495-1503.

Baskin CC, Baskin JM. Seeds, Ecology, Biogeography and Evolution of Dormancy, and Germination. (San Diego, USA: Academic Press), 1998.

Berman JD. Chemotherapy for leishmaniasis: biochemical me chanisms, clinical efficacy, and future strategies. Rev Infect Dis. 1998;10:560-586.

Boulikas T. Molecular mechanisms of cisplatin and its liposomally encapsulated form, Lipoplatin™. Lipoplatin™ as a chemotherapy and antiangiogenesis drug. Cancer Ther. 2007;5:351-376.

Bloor PA, Barker FS, Watts PC, Noyes HA, Kemp SJ. Microsa tellite Libraries by Enrichment. 2001. Available at: http://www.genomics.liv.ac.uk/animal/MICROSAT.PDF. (Acce -ssed January 2002).

Cabral PD, Soares TC, Lima AB, de Miranda FD, Souza FB, Goncalves LS. Genetic diversity in local and commercial dry bean (*Phaseolus vulgaris*) accessions based on microsatellite markers. Genet Mol Res. 2011;10:140-149.

Cai Y, Luo Q, Sun M, Corke H. Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. Life Sci. 2004;74:2157-2184.

Cheng ZX, Liu BR, Qian XP, Ding YT, Hu WJ, Sun J, Yu LX. Proteomic analysis of anti-tumor effects by Rhizoma Paridis total saponin treatment in HepG2 cells. J Ethnopharmacol. 2008;120:129-137.

Cheung JY, Ong RC, Suen YK, Ooi V, Wong HN, Mak TC, Fung KP, Yu B, Kong SK. Polyphyllin D is a potent apoptosis inducer in drug-resistant HepG2 cells. Cancer Lett. 2005;217:203-211.

Chinese Pharmacopoeia Commission. Pharmacopoeia of the People's Republic of China. (Beijing, China: China Medical Science and Technology Press), 2010.

Decrock E, Vinken M, De Vuyst E, Krysko DV, D'Herde K, Vanhaecke T, Vandenabeele P, Rogiers V, Leybaert L. Connexin-related signaling in cell death: to live or let die? Cell Death Differ. 2009;16:524-536.

Demir K, Bakir M, Sarikamis G and Acunalp S. Genetic diversity of eggplant (*Solanum melongena*) germplasm from Turkey assessed by SSR and RAPD markers. Genet Mol Res. 2010;9:1568-1576.

Deng S, Yu B, Hui Y, Yu H, Han X. Synthesis of three diosgenyl saponins:dioscin, polyphyllin D, and balanitin 7. Carbohydr Res. 1999;317:53-62.

Devkota KP, Khan MT, Ranjit R, Lannang AM, Samreen, Choudhary MI. Tyrosinase inhibitory and antileishmanial constituents from the rhizomes of Paris polyphylla. Nat Prod Res. 2007;21:321-327.

Dickson TC, Vickers JC. The morphological phenotype of b-amyloid plaques and associated neuritic changes in Alzheimer's disease. Neuroscience. 2001;105:99-107.

Dragland S, Senoo H, Wake K, Holte K, Blomhoff R. Several culinary and medicinal herbs are important sources of dietary antioxidants. J Nutr. 2003;133:1286-1290.

Dutta BK, Dutta PK. Potential of ethnobotanical studies in Northeast India: An Overview. Indian J Tradit Know. 2005;4:7-14.

Ellegren H. Microsatellites: simple sequences with complex evolution. Nat Rev Genet. 2004;5:435-445.

Ely JW, Kennedy CM, Clark EC, Bowdler NC. Abnormal uterine bleeding: a management algorithm. J Am Board Fam Med. 2006;19:590-602.

Fabricant DS, Farnsworth NR .The value of plants used in traditional medicine for drug discovery. Environ Health Perspect. 2001;109:69-75.

Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: Globocan 2008. Int J Cancer. 2010;127:2893-2917.

Fu YL, Zhao ZH, Shan YJ, Cong YW. Inducing effect of total steroid saponins from *Paris polyphylla* on platelet aggregation in vitro and its potential mechanism. Bulletin Academy Military Med Sci. 2007;31:416-419.

Fujimoto E, Sato H, Shirai S, Nagashima Y, Fukumoto K, Hagiwara H, Negishi E, Ueno K, Omori Y, Yamasaki H, Hagiwara K, Yano T. Connexin32 as a tumor suppressor gene in a metastatic renal cell carcinoma cell line. Oncogene. 2005;4:3684-3690.

Gao LL, Li FR, Jiao P, Yao ST, Sang H, Si YH. Apoptosis of human ovarian cancer cells induced by *Paris chinensis* dioscin via Ca^{2+}- mediated mitochondrion pathway. Asian Pac J Cancer Prev. 2011;12:1361-1366.

Gao LL, Li FR, Jiao P, Yang MF, Zhou XJ, Si YH, Jiang WJ, Zheng TT. *Paris chinensis* dioscin induces G2/M cell cycle arrest and apoptosis in human gastric cancer SGC-7901 cells. World J Gastroenterol. 2011;17:4389-4395.

Goncalves LS, Rodrigues R, do Amaral Junior AT, Karasawa M, Sudre CP. Heirloom tomato gene bank: assessing genetic divergence based on morphological, agronomic and molecular data using a Ward-modified location model. Genet Mol Res. 2009;8:364-374.

Grabley S, Thiericke R. Bioactive agents from natural sources: trends in discovery and application. Adv Biochem Eng Biotechnol. 1999;64:101-154.

Guo L, Su J, Deng BW, Yu ZY, Kang LP, Zhao ZH, Shan YJ, Chen JP, Ma BP, Cong YW. Active pharmaceutical ingredients and mechanisms underlying phasic myometrial contractions stimulated with the saponin extract from *Paris polyphylla* Sm. var. *yunnanensis* used for abnormal uterine bleeding. Human Reprod. 2008;23:964-971.

GuangLie C, WeiShi G, GaiLing H, JianPing C. Effect of paris Saponin on Antitumor and Immune Function in U14 Tumor-Bearing Mice. Afr J Tradit Complement Altern Med. 2013;10:503-507.

Hedge SN. Conservation of Northeast flora. Arunachal Forest News. 2000;18:10-22.

Hung JH, Lu YS, Wang YC, Ma YH, Wang DS, Kulp S.K, Muthusamy N, Byrd JC, Cheng AL, Chen CS. FTY720 induces apoptosis in hepatocellular carcinoma cells through activation of protein kinase C delta signaling. Cancer Res. 2008;68:1204-1212.

Jackman DM, Yeap BY, Sequist LV, Lindeman N, Holmes AJ, Joshi VA, Bell DW, Huberman MS, Halmos B, Rabin MS, Haber DA, Lynch TJ, Meyerson M, Johnson BE, Jänne PA. Exon 19 deletion mutations of epidermal growth factor receptor are associated with prolonged survival in non-small cell lung cancer patients treated with gefitinib or erlotinib. Clin Cancer Res. 2006;12:3908-3914.

Jain SK, Sumita S. Dictionary of Ethnoveterinary Plants of India. (New Delhi, India: Deep publications), p199, 1999.

Jiang H, Zhao P, Feng J, Su D, Ma S. Effect of Paris saponin I on radiosensitivity in a gefitinib-resistant lung adenocarcinoma cell line. Oncol Lett. 2014;7:2059-2064.

Ji Y, Fritsch PW, Li H, Xiao T, Zhou Z. Phylogeny and Classification of *Paris* (*Melanthiaceae*) Inferred from DNA Sequence Data. Ann.Bot. 2006;98:245-256.

Jun Z. Some bioactive substances from plants of West China. Pure Appl Chem. 1989;61:457-460.

Kala CP. Ethnomedicinal botany of the Apatani in the Eastern Himalayan region of India. J EthnobiolEthnomed. 2005;1:11.

Karthikiyan S. A statistical analysis of flowering plants of India. In: Flora of India Introductory Volume (Part 2), Singh NP, Singh DK, Hajra PK, Sharma BD ed. (Kolkata, India: Botanical Survey of India), pp. 201-217, 2000.

Kim EH, Yoon MJ, Kim SU, Kwon TK, Sohn S, Choi KS. Arsenic trioxide sensitizes human glioma cells, but not normal astrocytes, to TRAIL-induced apoptosis via CCAAT/enhancer-binding protein homologous protein-dependent DR5 up-regulation. Cancer Res. 2008;68:266-275.

Kobayashi N, Toyooka S, Soh J, Yamamoto H, Dote H, Kawasaki K, Otani H, Kubo T, Jida M, Ueno T, Ando M, Ogino A, Kiura K, Miyoshi S. The anti-proliferative effect of heat shock protein 90 inhibitor, 17-DMAG, on non-small-cell lung cancers being resistant to EGFR tyrosine kinase inhibitor. Lung Cancer. 2012;75:161-166.

Leal AA, Mangolin CA, do Amaral ATJ, Goncalves LS. Efficiency of RAPD versus SSR markers for determining genetic diversity among popcorn lines. Genet Mol Res. 2010;9:9-18.

Lecanu L, Rammouz G, McCourty A, Sidahmed EK, Greeson J, Papadopoulos V. Caprospinol reduces amyloid deposits and improves cognitive function in a rat model of Alzheimer's disease. Neuroscience. 2010;165:427-435.

Lee MS, Yuet-Wa JC, Kong SK, Yu B, Eng-Choon VO, Nai-Ching HW, Chung-Wai TM, Fung KP. Effects of Polyphyllin D, a Steroidal Saponin in, in Growth Inhibition of Human Breast Cancer Cells and in Xenograft. Cancer Biol Ther. 2005;4:1248-1254.

Liang SY, Soukup VG, Paris L. Flora of China Volume 24 Flagellariceae through Marantaceae. Wu ZY, Raven PH ed. (Beijing, China: Science Press and St Luis, USA: Bontanical Garden Press), pp. 88-95, 2000.

Li B, Yu B, Hui Y, Li M, Han X, Fung KP. An improved synthesis of the saponin, polyphyllin D. Carbohydr Res. 2001:331:1-7.

Li FR, Jiao P, Yao ST, Sang H, Qin SC, Zhang W, Zhang YB, Gao LL. *Paris polyphylla Smith* Extract Induces Apoptosis and Activates Cancer Suppressor Gene Connexin26 Expression. Asia Pac J Cancer Prev. 2012;13:205-209.

Long CL, Li H, Ouyang ZQ, Yang XY, Li Q, Trangmar B. Strategies for agrobiodiversity conservation and promotion: a case from Yunnan, China. Biodivers Conserv. 2003;12:1145-1156.

Mann J. Natural products in cancer chemotherapy: past, present and future. Nat Rev Cancer. 2002;2:143-148.

Martin PE, Evans WH. Incorporation of connexins into plasma membranes and gap junctions. Cardiovasc Res. 2004;62:378-387.

Miller KI, Qing C, Sze DMY, Neilan BA. Investigation of the Biosynthetic Potential of Endophytes in Traditional Chinese Anticancer Herbs. PLoS One. 2012;7:e35953.

Miyamoto J, Kurita S, Gu ZJ, Li H. C-Banding patterns in eighteen taxa of the genus Paris sensu Li, Liliaceae. Cytologia. 1992:57;181-194.

Mochida K, Shinozaki K. Advances in omics and bioinforma tics tools for systems analysis of plant functions. Plant Cell Physiol. 2011;52:2017-2038.

Morgan DO. Principles of CDK regulation. Nature. 1995;374:131-134.

Myers N, Muttermeier RA, Muttermeier CG, da Fonseca GAB, Kent J. Biodiversity hotspots for conservation priorities. Nature. 2000;403:853-858.

Nambara E, Nonogaki H. Seed biology in the 21st century: perspectives and new directions. Plant Cell Physiol. 2012;53:1-4.

Namsa ND, Tag H, Mandal M, Kalita P, Das AK. An ethnobotanical study of traditional anti-inflammatory plants used by the Lohit Community of Arunachal Pradesh, India. J Ethnopharmacol. 2009;125:234-245.

Norbury C, Nurse P. Animal cell cycles and their control. Annu Rev Biochem. 1992;61:441-470.

Oehler MK, MacKenzie I, Kehoe S, Rees MC. Assessment of abnormal bleeding in menopausal women: an update. J Br Menopause Soc. 2003;9:117-121.

Oliveira EC, Amaral Junior AT, Goncalves LS, Pena GF, Freitas Junior SP, Ribeiro RM, Pereira MG. Optimizing the efficiency of the touchdown technique for detecting inter-simple sequence repeat markers in corn (*Zea mays*). Genet Mol Res. 2010;9:835-842.

Oosterhout CV, Hutchinson WF, Wills DPM, Shipley P (2004). Micro-Checker: software for identifying and correcting genotyping errors in microsatellite data. Mol Ecol Notes. 2004;4:535-538.

Oyamada M, Oyamada Y, Takamatsu T. Regulation of connexin expression. Biochimica et Biophysica Acta. 2005;1719:6-23.

Ö zen T, Ç öllü Z, Korkmaz H. Antioxidant Properties of *Urtica pilulifera* Root, Seed, Flower, and Leaf Extract. J Med Food. 2010;13:1224-1231.

Patwardhan B. Drug Discovery and Development: Traditional Medicine and Ethnopharmacology. (New Delhi, India: New India Publishing Agency), pp.7-322, 2007.

Pande PC, Tiwari L, Pande HC. Ethnoveterinary plants of Uttaranchal- A review. Indian J Tradit Know. 2007;6:444-458.

Qi J, Zheng N, Zhang B, Sun P, Hu S, Xu W, Ma Q, Zhao T, Zhou L, Qin M, Li X. Mining genes involved in the stratification of *Paris Polyphylla* seeds using high-throughput embryo Transcriptome sequencing. BMC Genomics. 2013;14:358.

Raju J, Rao CV. Diosgenin, a Steroid Saponin Constituent of Yams and Fenugreek: Emerging Evidence for Applications in Medicine. In Bioactive Compounds in Phytomedicine. Rasooli I ed. (Rijeka, Croatia: InTech), 2012.

Ram VJ, Nath M. Current status of Chemotherapy of Leishmaniasis. Curr Med Chem. 1996;3:303-316.

Rastogi P, Mehrotra BN. Compendium of Indian Medicinal Plants. (New Delhi, India: National Institute of Science and Communication), pp. 479-480, 1993.

Ravipati AS, Zhang L, Koyyalamudi SR, Jeong SC, Reddy N, Bartlett J, Smith PT, Shanmugam K, Münch G, Wu MJ, Satyanarayanan M, Vysetti B. Antioxidant and anti-inflamma tory activities of selected Chinese medicinal plants and their relation with antioxidant content. BMC Complem Altern M. 2012;12:173.

Roa AC, Chavarriaga-Aguirre P, Duque MC, Maya MM, Bonierbale MW, Iglesias C, Tohme J. Cross-species amplifica tion of cassava (*Manihot esculenta*) (*Euphorbiaceae*) microsate llites: allelic polymorphism and degree of relationship. Am J Bot. 2000;87:1647-1655.

Riely GJ, Pao W, Pham D, Li AR, Rizvi N, Venkatraman ES, Zakowski MF, Kris MG, Ladanyi M, Miller VA. Clinical course of patients with non-small cell lung cancer and epidermal growth factor receptor exon 19 and exon 21 mutations treated with gefitinib or erlotinib. Clin Cancer Res. 2006;12:839-844.

Schlötterer C. The evolution of molecular markers - just a matter of fashion? Nat Rev Genet. 2004;5:63-69.

Schrager S. Abnormal uterine bleeding associated with hormonal contraception. Am Fam Physician. 2002;65:2073-2081.

Sharma PC, Grover A, Kahl G. Mining microsatellites in eukaryotic genomes. Trends Biotechnol. 2007;25:490-498.

Shino M, Watanabe Y, Umezawa K. Synthesis of N-substituted N-nitrosohydroxylamines as inhibitors of mushroom tyrosinase. Bioorgan Med Chem. 2001;9:1233-1240.

Shoemaker M, Hamilton B, Dairkee SH, Cohen I, Campbell MJ. *In vitro* anticancer activity of twelve Chinese medicinal herbs. Phytother Res. 2005;9:649-651.

Sordella R, Bell DW, Haber DA, Settleman J. Gefitinib-sensi-tizing EGFR mutations in lung cancer activate anti-apoptotic pathways. Science. 2004;305:1163-1167.

Sutter AP, Fechner H. Gene therapy for gastric cancer: is it promising? World J Gastroenterol. 2006;12:380-387.

Tag H, Das AK. Ethnobotanical notes on Hill Miri (Nyishi) tribes of Arunachal Pradesh. Indian J Tradit Know. 2004;3:80-85.

Tag H, Das AK, Pallabi H, Singh RK, Palit G. Botanical resources used in traditional wood carving industry among the Wancho tribe of Arunachal Pradesh. Indian J Tradit Know. 2008;7:148-156.

Tag H. Mainstreaming Conservation and Sustainable use of Medicinal Plant Diversity in Three Indian States. Final Technical Report submitted to UNDP Cell. (New Delhi, India: Govt. of India), pp. 1-124, 2012.

Tag H, Kalita P, Das AK. Biodiversity, Traditional Knowledge, IPR and rationalization of Traditional Phytomedicine in the Context of North East India. In Traditional Phytotherapy. Buragohain J ed. (New Delhi, India: Daya publishing house), pp. 27-46, 2013.

Tag H, Murtem G, Das AK, Singh RK. Diversity and distribution of ethno-medicinal plants used by the Adi Tribe in East Siang District of Arunachal Pradesh, India. Pleione. 2008;2:123-136.

Takhtajan A. A revision of Daiswa (*Trilliaceae*). Brittonia. 1983;35:255-270.

Tanaka M, Grossman HB. Connexin26 induces growth suppression, apoptosis and increased efficacy of doxorubicin in prostate cancer cells. Oncol Rep. 2004;11:537-541.

Talhouk R, Karam C, Fostok S, El-Jouni W, Barbour E. Anti-inflammatory bioactivities in plant extracts. J Med Food. 2007;10:1-10.

Terry RD, Masliah E, Salmon DP, Butters N, DeTeresa R, Hill R, Hansen LA, Katzman R. Physical basis of cognitive alterations in Alzheimer's disease: synapse loss is the major correlate of cognitive impairment. Annal Neurol. 1991;30:572-580.

Tiwari JK, Ballabha R, Tiwari P. New York Science Journal. 2010;3:123-126.

Tohda C, Kuboyama T & Komatsu K. Search for natural products related to regeneration of the neuronal network. Neurosignals. 2005;14:34-45.

Tohda C, Urano T, Umezaki M, Nemere I, Kuboyama T. Diosgenin is an exogenous activator of 1,25D3-MARRS/Pdia3/ERp57 and improves Alzheimer's disease pathologies in 5XFAD mice. Sci Rep. 2012;2:535.

Uprety Y, Asselin H, Boon E, Yadav S, Shrestha KK. Indigenous use and bio-efficacy of medicinal plants in the Rasuwa District, Central Nepal. J Ethnobiol Ethnomed. 2010;6:3.

Valdiya KS. Himalaya: Emergence and evolution. University Press Publ., Hyderabad, pp. 139, 2001.

Verma P, Mathur AK, Jain SP, Mathur A. In Vitro Conservation of Twenty-three Overexploited Medicinal Plants Belonging to The Indian Sub-Continent. Sci World J. 2012;929650:10.

Vieira EA, Carvalho FIF, Bertan I, Kopp MM, Zimmer PD, Benin G, Silva JAG, Hartwig I, Malone G, Oliveira AC. Association between genetic distances in wheat *(Triticum aestivum* L.) as estimated by AFLP and morphological markers. Genet Mol Biol. 2007;30:392-399.

Wang C, Schuller Levis GB, Lee EB, Levis WR, Lee DW, Kim BS, Park SY, Park E. Platycodin D and D3 isolated from the root of *Platycodon grandiflorum* modulate the production of nitric oxide and secretion of TNF-[alpha] in activated RAW 264.7 cells. Int J Immunopharmacol. 2004;4:1039-1049.

Wang L, Jia C, Yu Z, Liu X, Kang L, Cong Y, Shan Y, Zhao Z, Ma B, Cong Y. Pennogenin Tetraglycoside Induces Rat Myometrial Contraction and MLC20 Phosphorylation via PLC-IP3 and RhoA/Rho Kinase Signaling Pathways. PLoS ONE. 2012;7:e51536.

Watanabe T, Rajbhandari KR, Malla KJ, Yahara S. A handbook of medicinal plants of Nepal Japan. AYUR SEED. (Tokyo, Japan:Life Environmental Institute), 2005.

Workman P: Altered states: selectively drugging the Hsp90 cancer chaperone. Trends Mol Med. 2004;10:47-51.

Wu SS, Gao WY, Duan HQ, Wei J. Advances in studies on chemical constituents and pharmacological activities of Rhizoma Paridis. Chinese Traditional and Herbal Drugs. 2004;35:344-347.

Yina X, Qua C, Lib Z, Zhaic Y, Caoa S, Lina L, Fenga L, Yana L and Nia J. Simultaneous determination and pharmacokinetic study of polyphyllin I, polyphyllin II, polyphyllin VI and polyphyllin VII in beagle dog plasma after oral administration of Rhizoma Paridis extracts by LC-MS-MS. Biomed Chromatogr. 2013;27:343-348.

Yue G, Wei J, Qian X, Yu L, Zou Z, Guan W, Wang H, Shen J, Liu B. Synergistic Anticancer Effects of Polyphyllin I and Evodiamine on Freshly-Removed Human Gastric Tumors. PLoS One. 2013;8:e65164.

Zhang L, Ravipati AS, Koyyalamudi SR, Jeong SC, Reddy N, Smith PT, Bartlett J, Shanmugam K, Münch DG, Wu MJ. Antioxidant and Anti-inflammatory Activities of Selected Medicinal Plants Containing Phenolic and Flavonoid compounds. J Agr Food Chem. 2011;59:12361-12367.

Zhang J, Wang Y, Zhang J, Ding Y, Yu H, Jin H. Evaluation of mineral element contents in *Paris polyphylla var. yunnanensis* from Southwest China. Afr J Pharm Pharmacol. 2011;5:1792-1796.

Zhang W, Zhang D, Ma X, Liu Z, Li F, Wu D. Paris saponin VII suppressed the growth of human cervical cancer Hela cells. J Med Res. 2014;19:41.

Zhang X, Cui Y, Huang J, Zhang Y, Nie Z, Wang L, Yan B, Tang Y, Liu Y. Bioorg Med Chem Lett. 2007;17:2408-2413.

Zhao Y, Zhang J, Yuan T, Shen T, Li W, Yang S, Hou Y, Wang Y, Jin H. Discrimination of Wild Paris Based on Near Infrared Spectroscopy and High Performance Liquid Chromatography Combined with Multivariate Analysis. PLoS ONE. 2014;9:E89100.