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REVIEW ARTICLE

Ophiorrhiza, a promising herbaceous source of the anticancer compound camptothecin

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ABSTRACT
Camptothecin is an important source for the synthesis of some of the major anti-cancer agents such as irinotecan and topotecan. Traditional source of camptothecin are prominently woody plants such as *Camptotheca acuminata* Decne. and *Nothopodytes nimmoniana* (Graham) Mabb., and the increasing demand for camptothecin leads to the level of threatening their existence. *Ophiorrhiza* species composed of herbaceous plants with quick growth characteristics which are reported as alternative source of camptothecin. The present review focus on taxonomical status, traditional uses, biological activities and phytochemical constituents with a special attention in bioproduction of camptothecin from *Ophiorrhiza* species and its future prospects.

Introduction

Medicinal plants and their associated active pharmaceutical ingredients (API) are of much interest to the scientific and clinical researchers, not only because they are inexpensive but also due to minimal side effects compared to synthetic drugs (1). Some of the conventional drugs of plant origin include anti-malarial drug quinine from *Cinchona* sp. (2), anti-cancer drugs taxol from *Taxus brevifolia* Nutt. (3), vincristine and vinblastine from *Catharanthus roseus* (L.) G. Don. (4, 5), camptothecin from *Camptotheca acuminata* (6) and *Ophiorrhiza* spp. (7). The Southern western Ghats of India are endowed with rare and yet unknown species of plants and is counted among the top 25 biodiversity hotspots in the world. Many of the plants from the region, which are yet to be phytochemically characterized, have tremendous potential in therapeutic uses. Western Ghats region is considered as a rich repository of *Ophiorrhiza* species (8, 9).

Ophiorrhiza species are traditionally known as snake root because of its healing property against snake bite. Root of *O. mungos* and *O. japonica* are also traditionally used against snake bite, tumours and poisonous wounds. It also possesses anti-bacterial, anti-viral, anti-ulcer, anti-helminthic and anti-venom properties (10). The presence of camptothecin (CPT), a high value bioactive indole alkaloid endows anticancer property to this genus. Camptothecin and its derivatives were originally isolated from *C. acuminata* (6). Despite its increasing demand, camptothecin is still produced unsustainably by harvesting intact plants, mainly *C. acuminata* and *N. nimmoniana* (11). As the natural habitats of the species are becoming endangered owing to the uncontrolled exploitation of these plant species for camptothecin, it may become critical to develop alternative sources of camptothecin. *Ophiorrhiza* species is a good additional source for camptothecin because of the herbaceous nature and viability in *in vitro* tissue culture system (12–14). Other important phytochemicals, including derivative of CPT, pumiloside, luteolin, harman, tetrahydro alastonine, bracteatine, blumeanine, strictosidinic acid and lyalosidic acid are also present in varying quantities in certain species of *Ophiorrhiza* (15). Exploration for new species of *Ophiorrhiza* is very important to identify more potent medicinal sources. In this review, the importance of *Ophiorrhiza* species, their distribution status, phytochemical and pharmaceutical aspects with special interest on camptothecin and its future prospects are discussed.

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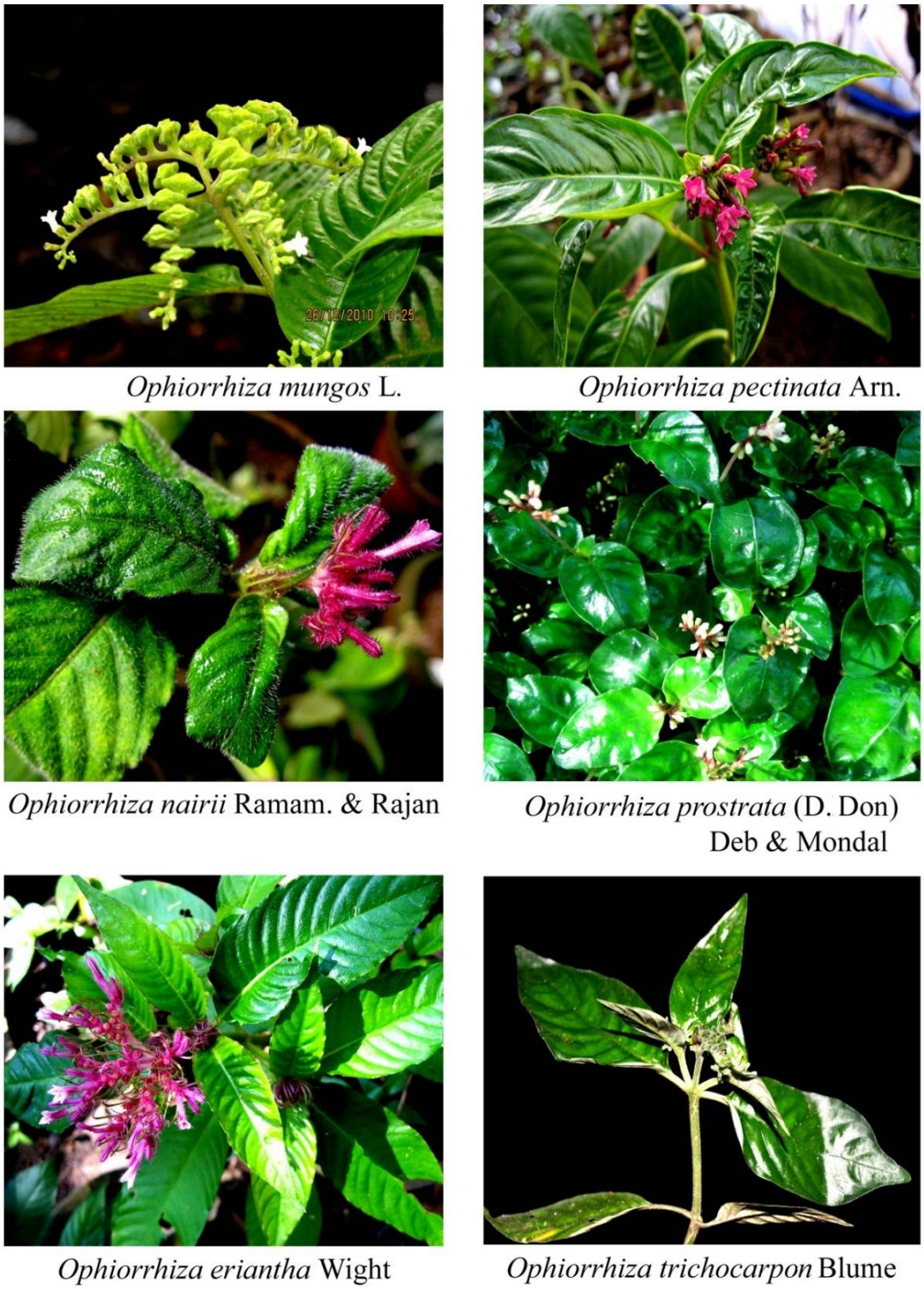

Fig. 1. Major species of *Ophiorrhiza* (*O. mungos* L., *O. pectinata* Arn., *O. nairii* Ramam. & Rajan, *O. prostrata* (D. Don) Deb & Mondal, *O. eriantha* Wight, *O. trichocarpon* Blume) found in the Western Ghats of Kerala.

Taxonomy

Ophiorrhiza belongs to the dicotyledonous family Rubiaceae and is represented by 150 species, distributed mainly in the Indo-Malay region (16). The greatest diversity of the genus *Ophiorrhiza* is observed in New Guinea and south eastern Asia (17). Therefore, the former is considered as the centre of origin of the genus (18). Some important *Ophiorrhiza* species are presented in Fig. 1. In India 48 *Ophiorrhiza* species and 6 varieties have been reported (10), among which 23 are endemic to the region. Majority of the species are distributed in Western Ghats, north-eastern Himalayas and the eastern states of India (Table 1). Western Ghats of Kerala are considered as a rich repository of *Ophiorrhiza* as evidenced by the survey reports showing the distribution of 24 species and 2 varieties, of which 13 species are endemic (19-23). Most of the species from north-eastern parts India have closer affinity with those of south-east Asia as compared to the peninsular Indian species (10). Two species namely, *O. mungos* and *O. rugosa* are distributed all over India.

Table 1. List of *Ophiorrhiza* species and varieties reported from Indian subcontinent

Sl. No.	Species and varieties	Distribution/Status
1	*O. barberi* Gamble	Kerala, Tamil Nadu (endangered, endemic to Southern Western Ghats)
2	*O. barnesii* Fischer	Kerala (critically endangered, endemic to Southern Western Ghats)
3	*O. borii* Deb & Mondal	Nagaland
4	*O. brunonis* Wight & Arn. var. *brunonis*	Kerala, Tamil Nadu, Karnataka (endemic to Southern Western Ghats)
5	*O. brunonis* Wight & Arn. var. *johnsonii* Hook. f.	Kerala (critically endangered, endemic to Southern Western Ghats
6	*O. caudata* Fischer	Kerala (critically endangered, endemic to Southern Western Ghats)
7	*O. caudipetala* Deb & Mondal	Meghalaya
8	*O. chandrasekharanii* Subba Rao & Kumari	Andhra Pradesh (endemic to Eastern Ghats)
9	*O. codyensis* Gamble	Karnataka (endemic to Southern Western Ghats)
10	*O. eriantha* Wight	Kerala, Tamil Nadu (endemic to Southern Western Ghats)
11	*O. fasciculata* D. Don	Western Himalaya, Sikkim, West Bangal, Orissa
12	*O. gracilis* Kurz	Nagaland
13	*O. grandiflora* Wight	Kerala, Tamil Nadu (endemic to Southern Western Ghats)
14	*O. griffithii* Hook. f.	Nagaland, Myanmar
15	*O. heterostyla* Dunn	West Bengal, Arunachal Pradesh, Myanmar
16	*O. hirsutula* Wight ex Hook. f.	Kerala, Karnataka, Tamil Nadu, Andhra Pradesh, Myanmar
17	*O. hispida* Hook. f.	Assam, Meghalaya, Myanmar
18	*O. incarnata* Fischer	Kerala (critically endangered, endemic to Southern Western Ghats)
19	*O. lurida* Hook. f.	Sikkim, Manipur, Eastern Tibet, South West of China
20	*O. mungos* L.	Kerala, Tamil Nadu, Sikkim, Nepal, Myanmar, Malay, Sumatra and Java, Sri Lanka
21	*O. munnarensis* Fischer	Kerala (critically endangered, endemic to Southern Western Ghats)
22	*O. mussaendiformis* Deb & Mondal	Meghalaya
23	*O. nicobarica* Balakr.	Great Nicobar Island
24	*O. nutans* Clarke ex Hook. f.	West Bengal, Sikkim, Nepal, Bhutan, Myanmar
25	*O. nairii* Ramam. & Rajan	Kerala (endemic to Southern Western Ghats)
26	*O. ochroleuca* Hook. f.	West Bengal, Sikkim, Myanmar
27	*O. oppositiflora* Hook. f.	Throughout Eastern India, Myanmar
28	*O. pauciflora* Hook. f. var. *glabra* Deb & Mondal	Arunachal Pradesh
29	*O. pauciflora* Hook. f. var. *pauciflora*	Arunachal Pradesh, Meghalaya
30	*O. pectinata* Arn.	Tamil Nadu, Kerala, Sri Lanka
31	*O. pykarensis* Gamble	Tamil Nadu (critically endangered, endemic to Southern Western Ghats)
32	*O. radicans* Gardn. ex Thw.	Kerala, Sri Lanka
33	*O. repens* Wall. ex G. Don	Assam, Meghalaya, Arunachal Pradesh, Myanmar
34	*O. rosea* Hook. f.	West Bengal, Sikkim, Myanmar, Thailand, Malay, Sumatra
35	*O. roxburghiana* Wight	Kerala, Tamil Nadu (vulnerable, endemic to Southern Western Ghats)
36	*O. rugosa* Wall. var. *argentea* Hook. f. Deb & Mondal	Karnataka, Sikkim, Meghalaya and all over Eastern Ghats of India, Andaman and Nicobar Islands, Nepal, Myanmar, Malay, Sri Lanka
37	*O. rugosa* Wall. var. *prostrata* D. Don	Kerala, Tamil Nadu, Bihar, Orissa, Maharashtra, Goa, Nepal, Bhutan, Malay, Sri Lanka
38	*O. rugosa* Wall. var. *rugosa* Hook. f.	Sikkim, West Bengal, Meghalaya, Nagaland
39	*O. rugosa* Wall. var. *decumbens* Gardn. ex Thw.	Kerala, Sri Lanka
40	*O. shendurunii* Shanavas *et al.*	Kerala (endemic to Southern Western Ghats)
41	*O. subcapitata* Wall. ex Hook. f.	Meghalaya
42	*O. succirubra* King ex Hook. f.	West Bengal, Sikkim, Meghalaya, Myanmar, Assam, Eastern India
43	*O. thomsonii* Hook. f.	West Bengal, Manipur, Nepal, Bhutan, Myanmar
44	*O. tingens* C. B. Clarke ex C. E. C. Fischer	Assam, Meghalaya, Nagaland, Myanmar
45	*O. tirunelvelica* Henry & Subram.	Tamil Nadu (endemic to Southern Western Ghats)
46	*O. treutleri* Hook. f.	West Bengal, Sikkim, Nepal, Myanmar
47	*O. trichocarpon* Bl.	Kerala, West Bengal, Orissa, Andaman Islands, Bangladesh, Myanmar, Thailand, Malay, Java
48	*O. villosa* Roxb.	Tripura, Andaman and Nicobar islands, Bangladesh, Myanmar, Malay
49	*O. wallichii* Hook. f.	Meghalaya, Nagaland, Arunachal Pradesh, Myanmar
50	*O. wattii* Fischer	Meghalaya, Nagaland, Manipur (endangered)
51	*O. wattii* Fischer var. *talevalliensis* Pal & Giri	Arunachal Pradesh
52	*O. rarior* H. S. LO	Kerala
53	*O. mycetiifolia* H. S. LO	Kerala
54	*O. sahyadriensis* Hareesh, V. B. Sreek. & K. M. P. Kumar	Kerala

Traditional uses

The term *Ophiorrhiza* has been derived from the Greek words '*Ophis*' meaning snake and '*rhiza*' root (10). Traditionally the root of *O. mungos* and *O. japonica* are used against snake bite (24). The root is also antihelmintic and alexipharmic (7). The whole plant or the roots are recommended for the

Table 2. Chemical constituents reported from *Ophiorrhiza* spp.

Sl. No.	*Ophiorrhiza* spp.	Chemical constituents reported	Ref.
1	*O. accuminata* L.	Palicoside, lyalosidic acid, palicoside methyl ester, harman	(42)
2	*O. discolor* R. Br. ex G. Don	Tetrahydroalastonine	(43)
3	*O. filistipula* Miq.	7-Methoxy camptothecin, camptotheicn, normalindine, strictosidinic acid	(44, 45)
4	*O. japonica* Blume	Harman, 6-hydroxy harman, lyaloside, lyalosidic acid, 10 hydroxylyalosidic acid, ophiorine A and B, ophiorine A and B methyl ester, pumiloside, deoxypumiloside, strictosamide, camptothecin, hydroxycamptothecin, friedelin.	(46-48)
5	*O. kuroiwae* Makino	Camptothecin, 9-methoxy camptothecin, harman, lyalosidic acid, ophiorine A and B.	(49}
6	*O. major* Ridl.	Bracteatine, ophiorrhizine	(31)
7	*O. pumila* Champ. ex Benth.	Camptothecin, strictosidine, 9-methoxycamptothecin, 10-hydroxy-camptothecin,strictosamide, 3-*(S)*-pumiloside, 3-(S)-deoxypumiloside,3-*(R)*-deoxypumiloside, chaboside, strictosidinic acid, lucidin3-O-β-purimeveroside, 3-hydroxy,2-hydroxymethylanthraquinone, 1-hydroxy2-hydroxymethy l-3 hethoxyanthraquinone, 3-*O*-caffeoylquinic acid, 9-β-glucosyloxy-camptothecin, pentaacetate,1,3-dihydroxy-2-hydroxymethyl-anthraquinone, 2-methylanthraquinone, 2-hydroxy-3-methyl-anthraquinone, 2-hydroxy-3-hydroxy- methyl anthraquinone, 1-hydroxy-2-hydroxymethyl-anthraquinone, 1,3-dihydroxy-2-methoxymethyl-anthraquinone, 1-hydroxy-2-methyl-anthraquinine, 1-hydroxy-2-hydroxy-methyl-3-methoxy anthraquinone, 3-hydroxyantraquinone-2-carbaldehyde, 2-n-butoxymethyl-1,3-dihydroxy-anthraquinone, tetraacetylsweroside, inamoside, 3-O-caffeoylquinic acid, penta-O-acetyl-loganin, β-sitosterol, 3-O-β-D-glucosyl- β-sitosterol, cholesterol, phaeophytin a, phaeophytin b.	(32, 50, 51, 52, 53, 54, 55, 56, 57)
8	*O. hayatana* Ohwi	Ophiohayatone A,B,C and D, 1 methyl-9*H*carboline 3-carboxylic acid, norharman, 6-hydroxyharmane, pumiloside, strictosamide, lyalosidic acid, lyalosidic acid, 9H-β-carboline-1-carboxylic acid, 1-methoxycarbonyl- β-carboline, 1-methyl-9-carboline-3-carboxylic acid, maxonine, lyaloside, desoxycordifolinic acid, vincoside, 2-hydroxymethyl-3-methoxyanthraquinone, methyl-p-hydroxycinnamate, methyl apraben, vanillin, ophiorridin-C, p-hydroxybenzaldehyde, ophiorridin-A, nonadecylferulate, ophiorridin-B, scopoletin, umbelliferone, 13^2-hydroxy-(13^2-R)-phaeophytin-a, aristophyll-c, methyl (10S)-hydroxypheophorbide-a, 13^2-hydroxy-(13^2-S)-phaeophytin-a, ursolic acid, adenine, nicotinamide.	(58)
9	*O. mungos* L.	Camptothcin, hydroxyl camptotheicin, 10-methoxy camptothecin, 9-methoxy camptothecin, luteolin-7-*O*-Glucoside, 5α-ergosterol-8(14) - ene-3β-ol, 5α-ergosterol-7-ene- 3β-ol.	(7, 28, 59)
10	*O. liukiuensis* Hayata	Pumiloside, deoxypumiloside, strictosamide, demethylsecologanol, 3‴-*O*-Glucosylsenburiside II, sweroside, *epi*-vogeloside, loganic acid, loganin, swertiaside-A, scopoletin, hyperin, *(6S,9R)*-reoside, *(6R,7E,9R)*-9-hydroxy megastima-4,7-dien-3-one-9-*O*-*β*-D-glucoside, chlorogenic acid, ursolic acid, β- sitosterol, daucosterol, 2-methyl-1,3,7- trihydroxyanthraquinone, 1-hydroxy-3-methyl- anthraquinone, 2-methyl-1-3-6-trihydroxyanthraquinone, harman, norharman,ophiorrhizine-A, ophiorrhizine-B, strictosamide, lyalosidic acid, ophiorrhizinone-E,soranjidiol-1-methyl ether, ophiorrhizinone-C, ophiorrhizinone-Bb, 1,3-dihydroxy-2-hydroxy ethylanthqaquinone, ophiorrhizinone-D, 1-hydroxy-2-methyl- anthraquinine, 1,3-dihydroxy-2,8-dimethoxy-7-methyl- anthraquinone, 2,8-dihydroxy-1,3-dimethoxy-7-methyl- anthraquinone, 8-hydroxy-3-methoxy-7-methyl-1,2-methylenedioxy- anthraquinone, ophiorrhizinone-A, ophiorrhisin-A, ophiorrhizol A, inamoside, 3-O-caffeoylquinic methyl ester, sodium chlorogenate, quinic acid 3,4-di-O-caffeate, quinic acid 3,5-di-O-caffeate, ursolic acid.	(12, 60)
11	*O. blumeana* Korth.	Bracteatine, blueanine, ophiorrhizine, ophiorrhizine12- carboxylate	(61)
12	*O. communis* Ridl.	Isomalindine, isomalidine-16- carboxylate	(62, 63)
13	*O. tomentosa* Jack ex Roxb.	Strictosidinic acid	(62)
14	*O. ferruginea* Valeton	Isomalindine, malindine, dihydrocycloakagerine, 3,14-dihydrodecussine, tetrahydrocycloakagerine, dihydrocyclol-akagerine	(45)
15	*O. rosacea* Ridl.	Harman-2-oxide, harman, lyalosidic acid, tetrahydroalstonine, strictosidinic acid, valesiaschotamine, isovalesiaschotamine,	(64)
16	*O. kunstleri* King	19-Methyl 3-14-didehydro normalindine, palicoside	
17	*O. bracteata* Korth.	Bracteatine, ophiorrhizine	(65)

treatment of snake-bite and scorpion sting (24). A decoction of roots, leaves and bark of *O. mungos* is given as stomachic and leaves are used for ulcer treatment (25). *O. japonica* is commonly used against ulcers, poisonous wounds, leprosy (26) and as an emmenagogue in China (27). The bruised roots are used as an application for various forms of cutaneous eruptions. The leaves are said to possess alterative properties and the flowers are prescribed as a stimulant and cardiac tonic in rheumatism and heart ailments. Sri Lankans use the entire plant of *O. mungos* to treat snakebite, rabbis, cancer and also as

a bitter tonic (7, 28). The leaves and roots of *O. subcapitata* are used for facial blemishes. Decoction of leaves and roots mixed with water are used as wash and the decoction mixed with honey is used for fever, sore throat and tonsils (29). *O. communis* and *O. tomentosa* are used as poultice and *O. communis* is also used to treat cough (30). Traditional healers of Indonesia and West Sumatra use the poultice of fresh aerial parts of *O. major* to treat skin disorders, especially eczema (31, 32). People of Arunachal Pradesh use the fruits of *O. fasciculata* as food. Leaves of *O. harrisiana* are used in Bangladesh as tea to cure body as well as chest pain (33). In Nepal and Trans-Indus region, the roots are boiled with oil and used as a dye for wool and hair (34). In Nepal *O. filistipula* is used to treat scalp infections in children and entire plant of *O. fasciculata* is used to treat angular stomatitis (35).

Biological activities reported for the genus

Ophiorrhiza species are reported with wide spectrum of biological activities. The methanol extracts of fresh stem and leaves of *O. marginata* and *O. kunstleri* showed anti-nematode activity (36). The ethanolic extracts of root, stem and leaf of *O. mungos* showed antiviral activity, especially against the Herpes virus (7) and the root extract acts as an antidote to neutralize Russells viper venom in *in vitro* and *in-vivo* (37). Antioxidant and cytotoxic activities of different parts of *O. mungos* (38, 39) and *O. prostrata* (40) have been studied and later shown to possess high cytotoxic activity. Besides, the hexane extract of *O. mungos* var. *angustifolia* inhibits pathogenic bacteria and fungus (41).

Chemical constituents reported from the genus

Though 150 species of *Ophiorrhiza* have been reported worldwide, published reports revealed that only 17 species have been subjected to detailed investigation of their chemical constituents (Table 2), and 19 species and one variety have been screened for camptothecin (CPT). The major class of compounds reported from *Ophiorrhiza* species are the alkaloids (indole alkaloids and quinoline alkaloids), secoiridoid monoterpenes, sesquiterpenes, steroids, quinines and phenyl propanoids.

Camptothecin and its derivatives reported from *Ophiorrhiza* species

Among the different classes of chemical constituents reported from the genus *Ophiorrhiza*, camptothecin is the most important. CPT (Fig. 2) is an aromatic, planar, pentacyclic, monoterpene alkaloid with pyrano-indolizinoquinoline skeleton, isolated from the seeds of the Chinese ornamental tree *C. acuminata* (Family: Nyssaceae) (6). Camptothecin (molecular formula $C_{20}H_{16}N_2O_4$) has a quinoline nucleus, and is biogenetically derived from the indole alkaloid strictosidine through its lactam derivative strictosamide (66). Strictosidine is formed by condensation of tryptamine with secologanin, a monoterpene glycoside (67).

The worldwide market for camptothecin and its derivatives has been estimated at about US $ 750 million in 2002, which rose to US $ 1 billion by 2003 and has reached 2.2 billion US $ in 2008 (68). This shows gradual increase in the demand and market value for CPT over the years. The steadily increasing demand for CPT and the scarcity of known plants from natural sources have led the researchers to find new plant sources for CPT (69, 70). Several plants namely, *Mappia foetida* (Wight) Miers, *Merrilliodendron megacarpum* (Hemsl.) Sleumer (71), *Ervatamia heyneana* T. Cooke (72) and numerous *Ophiorrhiza* species have been reported to contain camptothecin. Among the different plant genera that are reported to be the source of camptothecin, the genus *Ophiorrhiza* is very important as its habit is herbaceous. *O. mungos* L. (7, 73, 74) *O. mungos* var. *angustifolia* (Thw). Hook. f., *O. grandiflora* Wight, *O. barberi* Gamble, *O. shendurunii* A. E. S Khan et al, *O. trichocarpon* Blume, *O. pectinata* Arn.(75), *O. plumbea* Craib, *O. fruitocosa* Hance, *O. harrisiana* Heyne, *O. ridleyana* Craib (76), *O. alata* (77), *O. rugosa* Wall. *v*ar. *decumbens* (Gardn. ex Thw) (78, 79), *O. eriantha* Wight (80), *O. filistipula* Miq. (45), *O. japonica* Blume (48), *O. kuroiwaii* Makino (49), *O. prostrata* D. Don (81), *O. pumila* Champ. ex Benth. (50) and *O. rugosa* Wall. (78) are the major camptothecin containing species. The anticancer activity of camptothecin was first discovered serendipitously in 1958 in the fruit extracts of *C. acuminata.* The compound possesses inhibitory activity against an enzyme DNA topoisomerase I (82). Structure activity relationship studies have proved that the hydroxyl lactone E ring is the most important requirement for the high topoisomerase I mediated cytotoxicity, followed by the pyridine D ring (83). Apart from its potential anticancer activity, camptothecin is also active against the fowl plague virus (84), *Trypanosomes leishmania* (85), the Human Immuno Deficiency Virus (HIV) and the equine infectious anaemia virus (86).

Camptothecin shows severe bladder toxicity and poor water solubility (87). Hence, more active derivatives with polar groups such as topotecan ($C_{23}H_{23}N_3O_5$) and irinotecan ($C_{33}H_{38}N_4O_6$) are semi-synthetically derived from camptothecin. Topotecan is presently indicated as a second-line therapy for advanced ovarian cancer and small cell lung cancer. Irinotecan is approved for use in the treatment of advanced colorectal cancer, both as first-line therapy in combination with 5-fluorouracil and as salvage treatment in 5-fluorouracil refractory disease (88). There are several camptothecin analogues (topotecan, irinotecan, 10-methoxy camptothecin, 11-hydroxy camptothecin, desoxy camptothecin, 20-hexanoyl-10-methoxy camptothecin, 7-ethyl-10 hydroxy camptothecin, 9-amino camptothecin) which are at various stages of clinical evaluation (89).

Analytical methods for isolation of camptothecin

Extraction and product recovery are crucial factors and the solvent selected for extraction is of prime importance. The preferred solvent system for the extraction of camptothecin is chloroform/methanol mixture (90). Microwave-assisted extraction methods require shorter time, lesser volume of solvents, possess higher extraction rate and produced better products with lower costs (91). By comparing various extraction methods such as stirring, ultrasonic, Soxhlet and microwave extraction for camptothecin from *Mappia foetida*, the microwave assisted

Table 3. *Ophiorrhiza* species reported to contain camptothecin and its derivatives

Sl. No.	*Ophiorrhiza* spp.	Camptothecin and its derivatives	Reference
1	*O. alata*	Camptothecin	(77)
2	*O. decumbens*	Camptothecin	(78, 79)
3	*O. erianta*	Camptothecin	(80)
4	*O. filistipula*	7-Methoxy camptothecin, camptothecin	(45)
5	*O. fucosa*	Camptothecin, 9-methoxycamptothecin	(76)
6	*O. grandiflora*	Camptothecin	(77)
7	*O. harrisiana*	Camptothecin, 9-methoxycamptothecin	(76)
8	*O. japonica*	Camptothecin, hydroxy camptothecin	(48)
9	*O. kuroiiwai*	Camptothecin, 9-methoxy camptothecin	(49)
10	*O. mungos*	Camptothecin, hydroxy camptotheicin, 10-methoxy camptothecin, 9-methoxy camptothecin	(80)
11	*O. mungos* var. *angustifolia*	Camptothecin	(75)
12	*O. pectinata*	Camptothecin	(75)
13	*O. plumbea*	Camptothecin	(76)
14	*O. prostrata*	Camptothecin	(83)
15	*O. pumila*	Camptothecin, 9-methoxycamptothecin, 10- hydroxy camptothecin	(67)
16	*O. ridleyana*	Camptothecin	(76)
17	*O. rugosa*	Camptothecin	(73)
18	*O. shendurunii*	Camptothecin	(75)
19	*O. trichocarpon*	Camptothecin	(19)

Table 4. *In vitro* cultures of *Ophiorrhiza* species analysed for CPT

Sl. No.	Species	Tissue analysed/ Yield of camptothecin (µg g^{-1} DW)										Reference
		Whole plant	Leaves	Shoot	Root	Fruits	*In vitro* shoot	*In vitro* root	Callus	Hairy root	Cells suspension cultures	
1	*O. liukiuensis*	-	-	-	-	-	-	-	-	83.0±27.4	-	(100)
2	*O. kuroiwai*	-	-	-	-	-	-	-	-	219.3±31.44	-	(106)
3	*O. rugosa* var. *decumbens*	4.20	0.20	2.00	24.00	-	0.311	3.00±0.01	10.00±1.00	90.0	-	(74, 75, 77)
4	*O. prostrata*	-	22.00 – 62.00	800	1600	1650		190 – 120	-	-	20±2 – 330±2	(77, 93, 99)
5	*O. alata* Craib	-	83		388	-	94.0	556	0.01814	745.0±5	-	(73)
6	*O. mungos*	188.6±12.3	-	-	-	-		17 0±0.5 – 432±5	40±3 – 100±2	-		(37, 94, 95)
7	*O. pumila*	300 – 510	300 – 400		1000	-	-	-	-	240 – 1000	-	(50, 101, 109)
8	*O. eriantha Wt.*	-	-	-	-	-	-	-	0.001 – 0.0485	-	-	(76)
9	*O. mungos* var. *angustifolia*						0.120±10 – 0.740±50					(110, 111)
10	*O. prostrata* D. Don						1.10±0.029					(115)

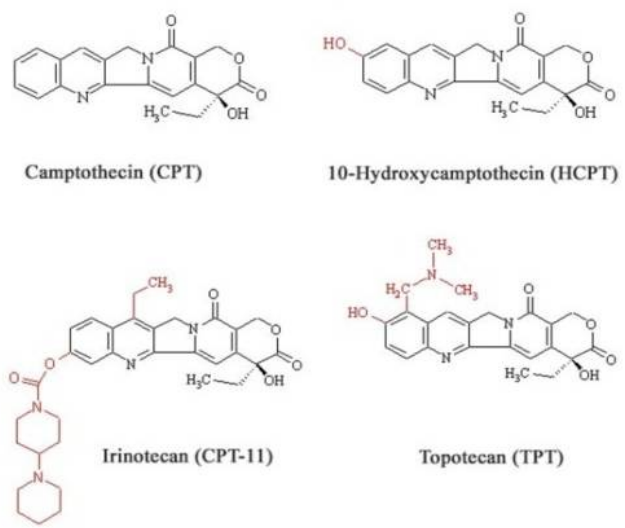

Fig. 2. Structure of camptothecin and its derivatives (68).

extraction was found to be more efficient (92). Different analytical techniques such as UV-visible spectrophotometer, high performance liquid chromatography (HPLC) and high performance thin layer chromatography (HPTLC) are used for the analysis of camptothecin in plant extracts. Presence of an aromatic ring system and quinoline chromophore, camptothecin exhibits characteristic UV absorbance and high fluorescence intensity (93), showing brilliant blue spots under UV 365 nm, making it easy to detect in TLC analysis. The absorption maximum of camptothecin at 256 nm is used for estimation purposes based on UV-visible spectroscopy (7, 74).

Though HPLC has been widely applied for the analysis of camptothecin and derivatives, recent reports revealed that HPTLC is emerging as more potential analytical tool for the studies of camptothecin and derivatives, due to the low operating cost, high sample throughput, sensitivity, reproducibility, accuracy and reliability (75). In the previous studies, different solvent systems were used *viz*. (i) ethanol: ethyl acetate (1:3 v/v) for *O. mungos* (7); (ii) ethanol: ethyl acetate: hexane (20:9.5:6.5:5.2 v/v) and chloroform: ethanol (15: 1 v/v) for *O. pumila* (94); (iii) chloroform: ethanol (24:1 v/v) for *O. rugosa*

(78); (iv) toluene: acetonitrile: glacial acetic acid (65:35:1 v/v/v) for *O. prostrata* and *O. mungos* (40). It was also reported as a precise reproducible solvent system (v) ethylacetate: chloroform: methanol (5:4.5:0.5 v/v) for the comparative study on the CPT content in different species of *Ophiorrhiza* genus (75).

Sophisticated analytical techniques like direct analysis in real time mass spectrum (DART- MS) and liquid chromatography mass spectrum/mass spectrum (LC-MS/MS) were used for the first time for CPT analysis (40). Previously, DART-MS, DESI-MS (desorption electrospray ionization mass spectrometry) and LC-MS had been used for the separation of CPT from *N. nimmoniana* and *C. acuminata.* DART-MS and DESI-MS are the new analytical techniques, which can be used in ambient conditions to obtain simple mass spectra, permits rapid qualitative and quantitative analysis of CPT (95).

Bioproduction studies

The extraction of CPT from raw materials is variable and consumes a lot of resources (96). Since natural regeneration of *Ophiorrhiza* is poor, tissue culture can be opted for the propagation of plantlets. Recently, CPT producing *Ophiorrhiza* plants have become viable alternative sources for CPT production by tissue culture (82, 97). *O. pumila* (67), *O. mungos* (98-100), *O. alata* (77), *O. liukiuensis, O. kuroiiwai* (12), *O. rugosa* (73), *O. rugosa* var. *decumbens* (79), *O. rugosa* var. *prostrata* (101), *O. japonica* (27), *O. Filistipula* (41) and *O. trichocarpon* (19) have been reported as rich sources of CPT and its derivatives by *in vitro* methods. For the production of camptothecin in large scale, several biotechnological approaches and published reports are available with different species of *Ophiorrhiza* for tissue culture multiplication and production of camptothecin (98-102). CPT yield in *in vitro* cultures of *Ophiorrhiza* species using different explants have been analysed (Table 3). Recent reports showed that the hormonal combination influences the production of CPT in *in vitro* cultures. Increased production of camptothecin was achieved by establishment of multiple shoot and root cultures of *O. rugosa* var. *decumbens*. The maximum amount of camptothecin in multiple shoot was 0.039%, whereas the shoots of plants grown in the field showed 0.002%. In adventitious root cultures camptothecin content were 0.065% and the roots of intact plants showed only 0.024% (97, 103). In *O. rugosa* var. *decumbens* albino plants seemed to be a good source of CPT and supplementation of 6 mg/l benzyl adenine (BA) was optimal for multiple shoot production in both albinos and green plants. Increasing BA concentrations showed elevated production of camptothecin yield in multiple shoots, and yield in albino shoots was higher compared to green shoots (78). *O. liukiuensis* an inter-species hybrid of *O. pumila* and *O. kuroiiwai* shows high amount of camptothecin production in *in vitro* than the parent species. Elicitation with methyl jasmonate in hairy root cultures showed slight enhanced production of CPT only in *O. liukiuensis* (12). Established both normal and hairy root cultures of *O. pumila* for mass production of camptothecin since intact plants showed lower production (104). A protocol for *in vitro* mass multiplication of plants through seedling cultures was established for *O. mungos* (112). *O. mungos* shoot cultures responded favourably in *in vitro* and produced higher amount of camptothecin when compared to naturally grown plants (74). Cell suspension cultures of *O. mungos* was established and elicitor mediated enhanced production of CPT along with high biomass was achieved (99). A high yielding cell line was selected from *O. mungos* and jasmonic acid mediated enhanced production of CPT was achieved in *O. mungos* (98). Adventitious root cultures of *O. mungos* were established from leaves derived from the *in vitro* shoots and proposed a modified medium for enhanced CPT production (100). The *in vitro* studies in *O. mungos* have shown that optimization of culture parameters and elicitation can enhance the CPT content in *in vitro* cell and organ cultures. In *O. prostrata* D. Don, at first tissue culture system was standardised (78), the adventitious root culture produced the higher amount (0.1%) of camptothecin. The seasonal variation in camptothecin content in plant parts of *O. prostrata* from natural habitats in northern Western Ghats have also been studied (81) (Table 4).

Development of new methods, such as, metabolic engineering involving key genes of CPT biosynthesis, promises alternative means for improvement of CPT content. So it is imperative to understand the details of the pathway for camptothecin biosynthesis. In a study, a construct harbouring the *ORCA3* gene, driven by the strong constitutive cauliflower mosaic virus (CaMV) 35S promoter was introduced into *C. acuminata* hairy roots to enhance the production of camptothecin (106). The gene for ORCA3, a jasmonate-responsive APETALA2 (AP2)-domain, is a transcription factor from *C. acuminata*, having similar TIA biosynthetic pathway. Previous study showed that overexpression of *ORCA3* can result in enhanced expression of several metabolite biosynthetic genes (such as *CPR*, *TDC* and *STR* etc.) and increased accumulation of terpenoid indole alkaloids in *C. roseus* (108). Another study described the cloning and characterization of cDNAs encoding strictosidine synthase and tryptophan decarboxylase, two key enzymes in the biosynthesis of TIA from hairy roots of *O. pumila* (54). Camptothecin is also obtained from plants regenerated from hairy roots. Because of the importance of CPT, the regeneration of hairy root derived plants is not only a fundamental study of camptothecin formation but also a system for feasible production (109). Genetically modified plants of *O. pumila* have been regenerated from its hairy root cultures, which yielded more than 1 mg g^{-1} dry weight of CPT and facilitated the release of relatively large quantities and scale-up production of CPT in a bioreactor. Suppression of camptothecin biosynthetic genes encoding tryptophan decarboxylase (TDC) and secologanin synthase (SLS), the two enzymes catalyzing the early steps in camptothecin biosynthesis, in the hairy roots of *O. pumila* was performed by RNA interference (RNAi), which resulted in the metabolic modification of secondary products in hairy roots of *O. pumila* (54).

Previous reports reveal that *in vitro* derived cultures have more CPT content than wild plants (78).

The hairy root cultures of *C. acuminata* and *O. pumila* produce camptothecin in the medium to large quantities. These reports suggest the possibility to develop large-scale production of CPT from *Ophiorrhiza* species. Besides plants, camptothecin has been reported in nature from the endophytic fungus isolated from the inner bark of *N. nimmoniana*, and studies utilising microbial techniques are in progress (110). Early reports revealed that geographical, ecological and climatic parameters influence CPT production in *C. acuminata* (111) and *N. nimmoniana* (112). Similar studies need to be carried out in *Ophiorrhiza* species for the selection of elite ones for the large scale production of CPT.

Future prospects

Very little work has been done on the anatomy, cytology, palynology and embryology of the genus *Ophiorrhiza*. Chromosome numbers has been reported as n=11 for *O. harrisiana* and *O. mungos* (113) and exceptional pollen development have been reported in *O. mungos* (113). Very little studies have been done on the phylogeny, chemical and evolutionary aspects of the genus (114). The Western Ghats in Kerala are the richest repository of *Ophiorrhiza* species and recently UNESCO declared Western Ghats as a heritage site (118). The forest in the Western Ghats has been severely fragmented due to various factors, including anthropological activities. Conservation, enhancement and sustainable utilization of plant resources are recognized as the vital segments in the natural resource management. Recent reports suggest that, *in vitro* multiple shoots produced more camptothecin than the wild plants in *Ophiorrhiza* species and numerous strategies have also been developed to improve the productivity of camptothecin such as medium optimization, cell line selection, cell immobilization, precursor addition, elicitation, genetic transformation, organ or hairy root cultures, metabolic engineering and integrated bioreactor engineering. Using these methods, coupling the large scale production of plants with high biomass can lead to enhanced bioproduction of camptothecin. Large scale multiplication of the plant through artificial seed method, enhanced production of CPT through irradiation and elicitation using both biotic and abiotic components are also promising fields to explore (116).

Conclusion

Camptothecin and its derivatives have received considerable attention due to the potential anticancer properties. Two semi-synthetic derivatives of camptothecin, topotecan and irinotecan are currently prescribed as anticancer drugs. The biological activity of the products of *Ophiorrhiza* has been well documented and the active ingredients such as camptothecin have been well characterized. Published reports on several scientific findings including the presence of camptothecin within the different species of the genus *Ophiorrhiza* emphasised the significance of the genus and its several species for the extraction of camptothecin and other new bioactive molecules. Also indicate that *in vitro* works in many *Ophiorrhiza* species, especially *O. mungos* (98–100), *O. prostrata* (116), *O. mungos* var. *angustifolia* (112, 117), *O. rugosa* (14) and *O. trichocarpon* Blume (13) have shown that the genus is a prospective one for CPT production after proper optimization studies. Due to high demand for camptothecin together with severe pressure on natural source have resulted in the depletion of natural plant populations. Therefore, more efforts must be made for alternate methods, such as tissue culture, hairy root culture, biotechnological approaches etc. for producing plants and camptothecin in a sustainable manner.

Authors' contributions

KG, DKP, SCV, DSN, GG and RKB collected the data; KG, DKP and SCV prepared the draft; KG, SCV and GG revised the manuscript; KG submitted the manuscript; KSK conceived the work, helped to analyze the data and revised the manuscript; RKB helped to execute phytochemical part; KPN provided scientific advice and critically reviewed the manuscript.

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Competing interests

Authors do not have any conflict of interests to declare.

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