**BIOPHYTUM REINWARDTII AND BIOPHYTUM VELDKAMPII: A REVIEW ON REVOLUTIONARY THERAPEUTIC AGENTS**

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**ABSTRACT**

**Background:** As plants have great ability to cure life threatening conditions, research works on plants has increased globally. In India, large numbers of plants are used traditionally to treat different ailments, but only few are validated. Among the *Biophytum* species, *Biophytum sensitivum* is the most widely studied one and only few studies have been carried out in *Biophytum reinwardtii* and *Biophytum veldkampii*. **Methods:** Previously published articles about *Biophytum reinwardtii* and *Biophytum veldkampii* have been collected and reviewed. **Observations:** *Biophytum veldkampii* differs from *Biophytum reinwardtii* in eglandular inflorescence and calyx, shorter pedicels than calyx and sepals exceeding capsules. Preliminary phytochemical analysis of ethanol extract of these species revealed presence of alkaloids, phenols, flavonoids, glycosides, cardiac glycosides, tannins, phlobatannins, quinones, anthraquinones, coumarins and steroids. A potent biflavonoid amentoflavone has been isolated in these two species and its anti-cancer activity was demonstrated. It is known to have antioxidant, antiviral, pain relieving, anti-inflammatory, antidepressant activity and antidiabetic activity. About 7-20 compounds were identified in GC-MS analysis, with major component identified as Gamma sitosterol. In cytotoxicity studies, leaves of *Biophytum veldkampii* and stem of *Biophytum reinwardtii* exhibited high efficacy. These species demonstrated high anti-cancer efficacy, with maximum activity against breast cancer cell line by *Biophytum reinwardtii*. A new flavonoid diglycoside from...
Biophytum reinwardtii demonstrated substantial antioxidant and anticancer activity.

**Conclusion:** To conclude, extensive research work needs to be implemented in these species to explicate chemistry, pharmacological activity and therapeutic applications as it is widely used in traditional practice to treat different ailments.

**KEYWORDS:** Biophytum veldkampii, Biophytum reinwardtii, Amentoflavone, anticancer.

**INTRODUCTION**
Recently research on plants has increased globally due to their great capacity to cure life threatening disease. Researches on medicinal plants have shown their therapeutic potential against various ailments. The efficacy of the plants can be explained on the basis of bioactive compounds present in them which forms the basis for several traditional medicinal systems. Discovery of drugs from herbs is gaining popularity because of increase in rate of advancement of disease. Compared to synthetic drugs plant derived products are non-toxic, clinically effective, inexpensive with less or no side effects. In India, traditionally numbers of plants are used to manage various disease conditions, but only few plants have been scientifically validated.[1]

The genus *Biophytum* has about eighty species of herbaceous annuals or perennials. It is found in tropical Asia, Africa, America and Philippines. In India it is noticed in the warm regions during the rainy season. Around eighty species are stated to be disseminated in the tropical and subtropical regions globally.[2] In India, seventeen species of this genus is stated to be present.[3] In Kerala, nine species is reported to be existing.[4] It is one among the “Dasapushpas” that includes ten holy flowering plants, which has an important role traditionally in Kerala.[5] It is an important medicinal plant in the Indian traditional system of medicine like Ayurveda. This plant has been used in the management of several disease conditions. These plants have been used traditionally for a very long period which shows their medicinal value. It has been used to clear the uterus after child birth. Also used for managing massive bleeding that occurs in women, hence called "Teendanaazhi". The plant is used for managing various painful conditions like leg cramps, leg pain, back pain, arthritis, degenerative joint and neck disorders, cervical spondylosis, osteo arthritis and rheumatoid arthritis. Other conditions in which it is useful are in convulsions, inflammatory tumors, bone spur and chest complaints. It is used as a tonic and stimulant.[6,7,8]
The objective of the current review is to provide updated knowledge and recent informations about two species of *Biophytum* namely *Biophytum reinwardtii* (Zucc.) Klotzsch., and *Biophytum veldkampii* Shanavas et al.

**BIOPHYTUM SENSITIVUM**

Among the genus *Biophytum*, the only species which has been studied in detail is *Biophytum sensitivum*. It is an annual herb with unbranched, straight hairy stem. Height of the plant ranges from 2.5-25cm. Leaves are peripinnate, 3.8-12.7cm long and consists of 6-15 pairs of leaflets that varies in size and seen at the upper part of the stem. They are oblong, progressively increasing in size upward with 1.5cm length and apiculate at apex. Flowers are dimorphic, yellowish and crowded at apices of peduncles. Sepals are lanceolate and about 7mm in length. Fruit is a capsule, marginally exceeding the sepals and ellipsoid.

Isolated phytochemicals from the aerial parts of plant are amentoflavone and cupressoflavone (biflavones), isoorientin, 3’-methoxyluteolin 7-O-glucoside and luteolin 7-methyl ether (flavonoids) and 5-Caffeoylquinic acid and 4-Caffeoylquinic acid (acids). Flavonoids were isolated from leaves as well. Epicatechin has been isolated from roots.

Several studies have been carried out in this plant that revealed its therapeutic potential like antioxidant, antitumor, antiapoptotic, antiangiogenic, antibacterial and antifungal, antidiabetic, lipid lowering, analgesic, anti-pyretic, anti-inflammatory, antifertility, antirolithiatic, antiepileptic, antiulcer, anthypertensive, chemoproctective, radioprotective and wound healing effect.

Acute toxicity study of aqueous leaf extract was found to be non-toxic up to 300 mg/kg body weight in mice. Methanol extract of whole plant was safe up to a dose of 4000mg/kg and no mortality was detected during 24 hr period.

Herbal cream and gel formulation containing this plant extract was evaluated for its antibacterial effect and was found to be highly effective. A tablet formulation containing methanol extract of plant extract was evaluated for its antioxidant and anti-diabetic effect and was found to be effective. It is one among the sixteen herbs in a formulation for diabetes used in Ayurveda since 2002. This formulation used to prevent and treat diabetes and its...
complications has been patented. In a clinical study in 30 patients, this formulation enhanced glycemic control along with glibenclamide.[33]

**DISTRIBUTION AND MORPHOLOGICAL DESCRIPTION OF BIOPHYTUM REINWARDTII AND BIOPHYTUM VELDKAMPII**

*B. reinwardtii* (Fig.1) is titled for 19th century Dutch naturalist and biologist Caspar Reinwardt. It is found in Malaysia, Ceylon, South China, India and Himalayas to an altitude upto 1000 m. It is seen in the shade, on waste land, banks of river and under humid thickets. It looks like a miniature tree and is otherwise known as Reinwardt’s tree plant. It matures to only 6-15 cm height, and has vertical, hairy stem. It has compound leaves which are 5-7 cm long and crowded near the base. 14-20 pairs of leaflets are seen that are oblong, 1 cm long and stalkless. Flowers are yellow supported on a 5-7 cm long stalk. They arise in umbels of 3-7 flowers, at the top. Flowering occurs from October to december.[34]

*B. veldkampii* (Fig. 2) is titled in honour of biologist Dr. J F Veldkamp for his contribution for the awareness of family oxalidaceae. It is inherent to India, and appears like a miniature palm. It is typically present in wet lands of India, Tropical Africa and Asia. It occurs in waste lands, open thickets, at low and medium altitudes of 50-200 m, in the shades of trees and shrubs in deciduous forest of southern Western Ghats of peninsular India. Stem is simple and grows upto a length of 15 cm. Leaves occurs in pairs of 7-16, that are oblong and terminals obovate. Flowering and fruiting occurs throughout the year.

*Biophytum veldkampii* differs from *Biophytum reinwardtii* in the following

- Eglandular inflorescence and calyx
- Pedicels clearly shorter than calyx
- Sepals exceeding the capsules[35]

![Biophytum reinwardtii](image-url)
Scientific classification

*Biophytum* belongs to the

Kingdom - Plantae
Division - Tracheophyta
Class - Magnoliopsida
Order - Oxalidales
Family - Oxalidaceae
Genus - Biophytum
Species - veldkampii and reinwardtii

Vernacular names of Biophytum species

<table>
<thead>
<tr>
<th>Common name</th>
<th>Reinwardt’s tree plant (<em>Biophytum reinwardtii</em>)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malayalam:</td>
<td>Mukkanthi</td>
</tr>
<tr>
<td>Tamil:</td>
<td>Tintaanaalee, Nilaccurunki</td>
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<tr>
<td>Kannada:</td>
<td>Horamuchhaka, Jalapushpa</td>
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<tr>
<td>Telugu:</td>
<td>Jalapushpa, Attapatti, Chumi</td>
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<tr>
<td>Hindi:</td>
<td>Lajalu, Lakshmana, Zarer</td>
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<tr>
<td>Marathi:</td>
<td>Mothi lajwanti, Jharera, Lahanmulaka</td>
</tr>
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<td>Bengali:</td>
<td>Jhalai</td>
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<tr>
<td>Sanskrit:</td>
<td>Lajjalu, Jhullipuspa, Panktipatra, Pitapushpa, Vipareetalajjaalu</td>
</tr>
</tbody>
</table>
TRADITIONAL USES

Whole plant of *Biophytum* is reported to be useful in urinary calculi, hyperdipsia, stomach pain, wound, bilious fever, snake bite, asthma and insomnia. *Biophytum* leaves and roots are used for treating pyrexia. The plant is stated to have tonic and stimulant properties, used for inflammatory tumours, chest complaints, convulsions, cramps, and its ash for upset stomach. Excessive salivation in cattle can be stopped by dried, powdered whole plant of Biophytum. The leaves have astringent, antiseptic and diuretic property. Crushed leaves or their juice is applied on burns. Paste made out of leaves is used to cover cuts and injuries. Decoction made from leaves is used in asthma and phthisis. The mature leaves have insulin like activity and are suggested for diabetes. The whole plant possesses tranquilizing action, removes dandruff and also acts as hair tonic. In the state of Bihar, the leaves and roots are given for lithiasis, sleeplessness, pyrexia and gonorrhoea. In Philippines, seeds are powdered and used for wound healing along with butter to promote discharge of pus.\textsuperscript{38} Traditional uses of *Biophytum* species is given in Table 1.

Table 1: Traditional uses of *Biophytum* species.

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Plant parts</th>
<th>Route of administration</th>
<th>Diseases</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Decoction of whole plant as a tonic</td>
<td>Oral</td>
<td>Stomach pain, tumor, epilepsy, complaints of chest, sleeplessness, asthma</td>
</tr>
<tr>
<td>2.</td>
<td>Root</td>
<td>Oral</td>
<td>Kidney stone and Gonorrhoea</td>
</tr>
<tr>
<td>3.</td>
<td>Whole plant</td>
<td>Topical</td>
<td>Chronic disorders of skin, Inflammation\textsuperscript{39}</td>
</tr>
<tr>
<td>3.</td>
<td>Decoction of whole plant</td>
<td>Oral</td>
<td>Snake bite\textsuperscript{40}</td>
</tr>
<tr>
<td>4.</td>
<td>Powdered seeds and leaves</td>
<td>Topical</td>
<td>Wound healing\textsuperscript{41}</td>
</tr>
<tr>
<td>6.</td>
<td>Leaves</td>
<td>Oral</td>
<td>Diabetes\textsuperscript{42}</td>
</tr>
</tbody>
</table>

*Biophytum reinwardtii* and *Biophytum sensitivum* are seen in the same environment and thus are blended in drug collection and both are used in the similar way. They are utilised in several Ayurvedic formulations like *Chemparuthyadi tailam*.\textsuperscript{38}

PHYTOCHEMISTRY

In a study conducted by Sreeshma et al, preliminary phytochemical screening of ethanolic extract of these species showed the presence of various compounds like alkaloids, phenols, tannins, phlobatannins, quinones, anthraquinones, coumarins, flavonoids, saponins, glycosides, cardiac glycosides and steroids in the entire parts.\textsuperscript{39} A novel flavonoid
diglycoside, apigenin-4',5-O-β-D-Diglucoside was isolated from whole plant of *Biophytum reinwardtii*.\[^{43}\]

Amentoflavone has been isolated from these two species and characterised by spectral methods.\[^{44}\] GC-MS analysis of ethanolic extract of these two species revealed that around 7-20 compounds were present in them. Main compound present was Gamma sitosterol succeeded by n-Hexadecanoic acid. The recognized compounds have various pharmacological activities like liver protective, antitumor, pain relieving, anti-ulcer, antipyretic, antimicrobial, spermicide, antidiabetic, and cholesterol lowering properties. Individual parts of the plant contain several phytocomponents. Although, bioactive components like n- Hexadecanoic acid and Gammasitosterol are commonly found in nature, their extraction in huge amount from other plant sources is challenging. The study concluded that these phytochemicals could be extracted in appropriate quantity from these species as they are predominant in most of the places.\[^{45}\]

**CYTOTOXIC POTENTIAL OF *BIOPHYTUM* SPECIES**

The cytotoxic potential of different plant parts of *Biophytum* species was analyzed and it was found to be highly effective against Brine shrimp napauli when compared to the positive control potassium permanganate. The ethanolic extracts of *B. veldkampii* leaves and *B. reinwardtii* stem were found to be highly effective than other plant parts with an LC 50 value of 3.73 and 2.87µg/ml respectively, which is comparable to that of standard potassium permanganate (LC 50-3.92µg/ml). A gradual increase in lethality was observed with increase in concentration of plant extracts. The assay was found to be effective for scientific validation of two species of *Biophytum*.\[^{43}\]

The apoptotic effect of *Biophytum* species were studied by investigating changes on cell morphology in different cell lines. Nuclear condensation assay was performed in breast cancer cell lines, cervical cancer cell line and ovarian cancer cell line. The assay utilized two concentrations of plant extracts. The investigation shown that ethanolic extract of both the species show high anticancer efficacy against various cell lines studied. Maximum activity was shown against breast cancer cell line MCF-7 by *B. reinwardtii*. This was followed by cervical cancer cell line, breast cancer cell line MDA-MB-231 and ovarian cancer cell line. The apoptotic cells were distinguished from non-apoptotic cells by strong staining of the DNA in condensed chromatin of dead cells by fluorescent dyes. The percent of dead cells increased in a concentration dependant way in the presence of test sample.\[^{46}\]
FLORAL VOLATILES OF BIOPHYTUM SPECIES
A comparative study on floral volatiles of *B. reinwardtii* and *B. veldkampii* was carried out and it was found that *B. reinwardtii* flowers exhibited a constant and pleasing aroma. Also the phytochemical responsible for this pleasant smell was analysed. By the process of hydrodistillation the essential oils were extracted and the constituents were investigated by GC-MS analysis. The main component was found to be n-hexadecanoic acid in both the species. Some sequesterenes have been identified in essential oil of *B. reinwardtii* which was absent in flowers of *B. veldkampii*. The study concluded that, the sequeterenes found in flowers of *B. reinwardtii* may be responsible for its pleasant smell.\(^{[47]}\)

A research work was carried out by Varadarajan et al on *B. reinwardtii*. They isolated a new flavonoid diglycoside apigenin-4',5-O-β-D-Diglycoside from this plant. They also evaluated the antioxidant and anticancer effect of the isolated compound. Isolated compound was subjected to DPPH radical scavenging assay and FRAP assay which revealed significant antioxidant activity. The compound showed a concentration dependant effect by inhibition of DPPH radical and showed a better activity than the positive control BHA. In FRAP assay, the compound exhibited good ferric reducing activity which is equivalent with ascorbic acid but inferior to gallic acid. Anticancer activity was estimated in human cervical cell lines and human colon cell lines by MTT assay. A reduction in cell viability and increase in cytotoxicity was seen in a concentration dependent manner. The isolated compound was found to have substantial antioxidant and anticancer activities.\(^{[38]}\)

TOXICITY STUDIES
Acute toxicity and sub chronic toxic potential of alcohol extract of *B. reinwardtii* whole plant was carried out in white mice and rats. Different concentrations of the extracts were orally given and various parameters were examined like body weight, liver function test, blood test and absolute and relative weight of organs. After one month no substantial changes in these parameters were observed between test and control groups which showed that the plant is safe in animals.\(^{[48]}\)

Reported research studies in *Biophytum* species are given in Fig. 3
AMENTOFLAVONE

These two species revealed the presence of a potent bioactive compound amentoflavone (Fig. 4). It is a biflavonoid, which is reported to possess antioxidant, anti-inflammatory, analgesic, antiviral and antidepressant property. Quantification of amentoflavone in ethanolic extract of these species by HPLC method showed that highest amount was present in stem of *B. Reinwardtii* (17.18mg/gm extract) followed by root of *B. veldkampii* and *B. reinwardtii*. In other parts of plant, it was found to be present in less than 2mg/gm. The study stated that these species are rich in amentoflavone and extensive isolation is economically feasible as these species are commonly found in all habitats.\[49\]

Amentoflavone has been isolated from these two species which was characterised by various chromatographic and spectral methods. The yield of the compound was higher in *Biophytum reinwardtii* than *Biophytum veldkampii*. Also it was screened for its cytotoxic potential in different cell lines. The study result proved that amentoflavone obtained from these species is not toxic to normal cell lines, whereas it produced a dose dependant cytotoxic effect in cancer cell lines, as a decrease in cell proliferation was observed in MCF cell lines. It was confirmed by the method of flowcytometry. The study concluded that these plants are potent source of amentoflavone as they were obtained in sufficient amount.\[44\]
It is an active ingredient present in *Biophytum sensitivum*, also present in various plants like *Selaginella sinensis*, *Selaginella tamariscina*, *Cycas rumphii*, *Trifolium alexandrinum*, *Thuja orientalis*, *Antidesma laciniatum*, and *Tratinnickia rhoifoli*.

Feng W et al investigated the antidiabetic effect and probable mechanism of action of amentoflavone in diabetic mice. Diabetes was induced in mice by administering streptozotocin followed by administration of amentoflavone for eight weeks. On evaluation of biochemical parameters and glucose level, a significant improvement was observed. Also, it was found to increase the activity of glucokinase, phosphofructokinase and pyruvate kinase and inhibited the activity of glycogen synthase kinase, phosphoenolpyruvate carboxykinase and glucose-6-phosphatase. In skeletal muscles, it was found to increase the translocation of glucose transporter type 4 (GLUT4). The study concluded that amentoflavone exerted the antidiabetic effect by regulating fat and glucose metabolism probably through its anti-oxidant effect and stimulating P13K/AKT pathway.

Recent research works on amentoflavone revealed that, it is an effective antidiabetic agent and acts by inhibiting alpha glucosidase and alpha amylase enzymes. Suppression of these enzymes leads to decrease in glucose absorption and finally regulates blood sugar level. Ogunwa TH and Ayenitaju FC evaluated *in-silico* mode of action of amentoflavone to determine its antidiabetic activity through inhibition of human alpha amylase. Molecular docking was performed by Vina plugin in PYMOL 1.3. Their study results indicate that amentoflavone causes competitive inhibition of substrate binding site of alpha amylase and there by inhibits substrate access. In another study, Ogunwa TH evaluated the interaction of amentoflavone with alpha glucosidase and concluded that amentoflavone competitively inhibited the active site of protein.
CONCLUSION

*Biophytum sensitivum* is the most extensively studied species among *Biophytum* genus. Traditionally different plant parts of *Biophytum reinwardtii* and *Biophytum veldkampii* are used in the management of many disease conditions. They are identified to have extensive range of medicinal properties like antidiabetic, anticonvulsant, antiinflammatory, antirheumatic, antitumor, antiurolithiatic, antihypertensive, antibacterial and wound healing effect. Research works were carried out on these plants to prove their anticancer effect. GC-MS analysis of these species had shown the presence of around 20 compounds. Studies on cytotoxic potential showed that they are extremely active against brine shrimp napauli. Amentoflavone, a therapeutically potent compound was isolated from these species and its anticancer effect was demonstrated. A new flavonoid diglycoside has been isolated from *Biophytum reinwardtii*, which revealed high antioxidant and anticancer effect. Presence of some sequesterenes was identified to be the reason for peculiar fragrance of *Biophytum reinwardtii* flowers. The only pharmacological activity studied in detail in these species is anticancer effect and still needs to be screened for several pharmacological actions. The phytochemical examination revealed presence of several bioactive constituents, but no systematic effort has been made to isolate these components that have the potentiality to be developed as harmless and valuable medicines. From these data, it can be inferred that detailed research works are required to screen the different pharmacological actions as well as to isolate the bioactive components accountable for its diverse role in disease management.
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CONFLICT OF INTEREST
The authors declared no conflict of interest with respect to the authorship, research or publication of the article.

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