Floral morphology and reproductive biology of medicinal plants: Costus (Costaceae)

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Abstract
Floral morphological studies were conducted on Costus pictus D. Don and Costus speciosus (Koening) sm, belonging to family Costaceae. Flowering period was found to be from May to November. Inflorescence was born on a leafy shoot at a terminal position in both the species. Colour of bract is green in C. pictus and dark red in C. speciosus. Extra floral nectary is present on the bracts in form of vertical dark lines. A single petaloid labellum, mono petaloid fertile stamen and inferior apparent 2-locular ovary in C. pictus and 3-locular ovary in C. speciosus are found. The thin and thread-like style is positioned between the thecae of the fertile stamen. Two lobed stigma bears appendages. Placement is parietal in C. pictus and axile in C. speciosus. During the whole flowering period, ants were the only pollinators to visit the flower and inflorescence. The present study highlights the key for identification of the species and their pollinators.

Keywords: Appendage, bracts, Costus, inflorescence, ovary, petaloid

Introduction
The monocot family Costaceae belongs to order Zingiberales (Dahlgren et al. 1985) [5]. The genus Costus is the largest in the family with about 150 species tropical in distribution (Humphries 1985 and Hickey 1981) [10, 13]. The Shoot of Costus has a characteristic spiral monostichous phyllotaxy (Kirchoff and Rutishauser 1990) [17]. The floral structure of Costaceae is also unique in the order Zingiberales. Only a single fertile stamen develops while the infertile five stamens fuse to form a large, petaloid labellum that dominates the floral display (Kirchoff 1988; Troll 1928) [16, 32]. Costus igneus is commonly known as fiery Costus, step ladder, spiral flag, crepe ginger or insulin plant. Costus pictus D. Don is commonly known as American insulin plant (Fig. 1 A). Medicinally C. pictus D. Don leaves are used to control the blood sugar level and C. speciosus (Koening) sm rhizome is used to control the sugar. C. speciosus (Koening) sm is native of Malaya Peninsula of South East Asia (Rani, et al. 2012) [22]. In India, it is found in Central India, western ghats of Maharashtra, Karnataka and Kerala. C. pictus D. Don is native to Mexico but introduced to India a few years back (Shiny, et al. 2016) [28]. For evolution and survival, reproduction is a natural process of increasing the number of individuals of the same species. Detailed knowledge of reproduction biology is required for successful cultivation and conservation of plants (Moza and Bhatnagar 2007) [20] and also for the study of the phylogeny. Due to a gap in the knowledge of reproductive biology of these two species, this work is carried forward. The present study provides a detailed account of the reproductive biology of two species of Costus in relation to floral morphology. Different parts of the Costus are used in treating various diseases. Active Phyto-constituent is Diosgenin. The rhizomes are bitter and used mainly for treating diabetes. They show anti-helminthic, astringent, expectorant properties. The extract of the rhizome is employed for treating burning sensation, constipation, leprosy, asthma, bronchitis, anaemia and other skin ailments (Bown Deni, 2008) [3]. Rhizomes of Costus are used as herbal remedies for fever and its paste is employed for treating boils. It is also used to make sexual hormones and contraceptives (Warrier et al., 1994; Rastogi and Mehrotra, 1991) [23, 33]. Leaves are used for scabies and stomach ailments. Leaves are ground into a paste and applied to the forehead to bring down high fever. Besides rhizomes, stems also are used for treating blisters and burns. Roots are used against snakebite (Rathore and Khanna, 1978) [24].
Costus is traditionally used as a medicinal herb mainly for its tonic, stimulant, carminative, diuretic, digestive and antiseptic properties. The rhizome is used internally in the treatment of abdominal pain, chest pains, liver problems, jaundice, gall bladder pain etc. (Sivarajan and Balachandran, 1994) [10]. In Ayurveda Costus speciosus is employed to subdue Vata and Kapha and promotes complexion. It is reported to cure dyspepsia, fever, cough and other respiratory disorders. It is one of the constituents of indigenous drug, “amber mezhhu” useful in rheumatism (Chopra et al., 1956). [4]. The rhizome possesses antiferility, anticholinesterase, anti-inflammatory and anthelmintic activities (Hussain et al., 1992) [14]. An essential oil from rhizomes showed antimicrobial activity (Asolkar et al., 1992) [11]. Steroid saponins and sapogenins from Costus speciosus were reported to possess antifungal activity. The medicinal preparation further shows insulin potentiating action in addition to decreasing, blood glucose. Similarly, the leaves of Costus speciosus are known to have hypoglycaemic properties (Eliza et al., 2009) [6]. There is a need of research on Costus an important endangered medicinal plant.

Material and Methods
Costus pictus D. Don and Costus speciosus plants were collected from Dev Sanskriti University, Haridwar. The plants were grown in the College Germplasm Garden, Department of Botany, R.G.P.G. College, Meerut. The floral morphological investigations were recorded in the field and laboratory. Ten individuals of each species were observed on daily basis. Photographs were captured and slides were prepared. Floral visitors and pollinators were recorded during the entire flowering periods.

Results and Discussion
The field and lab work revealed the peculiar key characters of studied species (Table 1). It was noted that they are perennial with an erect or spreading stem. The plant reaches a height of about 60-90 cm. With the tallest stem falling over and lying towards the ground. The rhizome and shoot are composed of nodes and internodes. Leaves with sheathing leaf bases originate at the node. The leaf lamina comes out from the apex of the sheath. The leaf as well as a bract, emerge along with the shoot in the right handed spiral (Halle1967) [9]. Vegetative shoot terminates into a reproductive shoot or inflorescence in both the species of Costus. Thus shoot is homologous i.e. plant produces a single shoot type combining vegetative and reproductive portions (Fig: A-C). The other key observations are followings:

Inflorescence
In family Costaceae inflorescence terminates into a leafy shoot emerging from the rhizome or a shorter leafless shoot emerging from the rhizome or can be borne in the axil of a leaf. In our investigations in both the species, inflorescence terminates into a leafy shoot emerging from a rhizome (Homoplyadic) (Fig: B-C).

Bract
Near the apex of the bract a slightly thickened vertical line of dark colour surrounding bract is present. The cells of the vertical line or callus produce nectar. It is an extrafloral nectary (Maas et al. 2016) [19]. The nectar attracts ant that protects the inflorescence against oviposition by flies. This observation conforms with the observations of Schemske (1980-1982) [26, 27], according to his observations, these flies oviposit in immature fruit and their larvae destroy the seeds and arils of the plant resulting in seed loss. The transition between vegetative leaves and reproductive bract occur gradually, not abruptly. Lowest bract retains the character of leaf lamina as seen in C. pictus D. Don and C. speciosus (Koening) sm. Bract appendages are not found in both the species (Maas et al, 2016) [19] (Fig: D-E).

Bracteole
There is only one bracteole surrounding the flower. The colour of the bracteole is red.

Flowers
It was observed that flower is complete, zygomorphic, hermaphrodite (Fig: F-G).

Calyx
Colour of the calyx is the same as the bract and bracteoles in both the species. A Callus is found on calyx lobe. Callus secretes nectar.

Corolla
Flower bear 3-lobed corolla, a single petaloid sterile labellum.

Androecium
It is in form of one fertile petaloid stamen (Fig: H-I).

Labellum
It is a unique feature of the family. It is composed of five fused staminodes. It is the showy part of the flower. The lower part of the labellum is united with the stamen forming a second tube (Maas 1972) [18]. Similar structures have been reported in both the species. The Inner side of labellum in both the species have different colour “nectar guide” presumed to be used in pollination. The Labellum is funnel-shaped in C. pictus D. Don and somewhat horizontally flattened in C. speciosus (Koening) sm. It appears that horizontally flattened labellum provides a horizontal landing platform for a pollinator.

Gynoecium
It consists of stigma, style and ovary, tricarpellary, trilocular with axile placentation (Fig: J-O).

Stigma
Appendages are present on the bilobed funnel-shaped stigma. Observations reveal that in C. pictus D. Don Stigma has dorsal two-lobed appendage. In C. speciosus (Koening) sm stigma has a dorsal rounded appendage

Style
The thin style is positioned between the thecae of the fertile stamen. The style lies embedded between the two narrowly elliptic thecae forming the anther. Style is hooked between the apex of the theca by the appendage of stigma. Thus our observations regarding attachment of stigma and style with anther are correlated with the findings of Maas (2016) [19].

Ovary
There is the dissimilarity of the ovary in both the species studied. In C. pictus D. Don ovary appears to be bilocular although in all the species including C. speciosus (Koening) sm ovary is trilocular. In C. pictus D. Don third locule is suppressed. Thus ovary of C. pictus D. Don shows deviation from the characteristics of the family. In C. pictus D. Don
ovary appears to be bilocular where the third locule is suppressed.

**Placentation**

It is axile in family *Costaceae*. A similar observation is found in *C. speciosus* (Koening) sm. Placentation in *C. pictus* D. Don is parietal. It reveals that the situation of placentation is not so simple and it appears that there is a clear cut trend in the evolution of parietal placentation from basic axile placentation (Puri 1952) [21]. It is tempting to interpret the character as a trend in phylogeny.

**Floral biology and pollination**

According to literature studied, pollination can be insect or bird pollinated. According to Maas (2016) [19] and several workers, the main characters forming potential pollination syndromes is the colour and the texture of the flower and shape of the labellum. Nectar guide is a contrasting colour reddish blotch in *C. pictus* D. Don and creamy yellow blotch in white coloured *C. speciosus* (Koening) sm in the inner side of the labellum. Field observations regarding pollinators coming to flowers are only ants (Fig: P-Q). They followed the nectar guide towards the centre of the flower. Ants brush pollen from the fertile stamen into their back. When they enter another flower, pollen is transferred to the stigmatic surface of the subsequent flower. As the stigma is above the anther, it will facilitate cross-pollination. Floral visitors like birds and flies were not found to visit the flower for nectar and pollination. This can be the result of climate change and change in the habitat of the plant. During the survey, it was noted that pollinator ants come to the inflorescence and flower, where they get pollen and nectar as a reward. Comparative study of floral morphology of two species of *Costus* are shown in Table 1.

Not too much work is found in this valuable medicinal plant genus. So further studies on different species of genus on various aspects are required.

| Table 1: Comparative study of floral morphology of two species of *Costus* |
|-----------------------------|-----------------------------|
| **Parameter**               | **C. pictus D. Don**         | **C. speciosus (Koening) sm** |
| Inflorescence               | Terminal in position, compact, globose, green bract, length 8-9 cm, width 6-7 cm. | Terminal in position, ovoid, red bract, length 9.5-10 cm, width 6.5-8.0 cm. |
| Bract                       | Large green, ovate, length 2.5-3.0 cm, width 3.0-3.5 cm bract fibres absent, flowers arranged in a spiral in the axil of the bract, extra floral nectary present. | Bract large dark red, ovoid to globose, length 2.5-3.5 cm, width 0.8-1.0 cm, bract fibres absent, flowers arranged in a spiral in the axil of the bract, extra floral nectary present. |
| Bracteole                   | One enclosing each flower, length 1.8-2.2 cm. | One enclosing each flower, length 2.2-2.5 cm. |
| Flower                      | Zygomorphic, Yellow, membraneous with reddish stripes, with cup-shaped labellum. | Zygomatic, White crepe paper yellowish colour with cup-shaped labellum. |
| Calyx                       | 3 lobed, tubular, length 1.1-2.2 cm, colour basal white and apical part white. | 3 lobed, tubular, length 2.3-2.6 cm, colour basal white and apical part white. |
| Corolla                     | Yellow, strong, 3 lobed, oblong, labellum present. | White 3 lobed, Elliptic oblong, labellum present. |
| Androecium                  | Single fertile stamen, median on an oblong petaloid process. | Single fertile stamen, median on an oblong petaloid process. |
| Gynoecium                   | Ovary inferior, tricarpellary, Stigma Bifid, Style long & thin. Placentation is Parietal. | Ovary, inferior tricarpellary, Stigma crescent-shaped, style ciliate long & thin. Placentation is axile. |
| Flowering periods           | May to November              | May to November              |
| Medicinal Value             | Leaves are sour used to treat diabetes. Diosgenin is the active constituent. | Rhizome used to treat diabetes. Diosgenin is the active constituent. Leaves are bitter. |

Fig 1: Plate 1- Fig A: Spiral phyllotaxy of *C. pictus* and *C. speciosus*; B & C: *C. pictus* and *C. speciosus* showing emergence of reproductive shoot; D & E: *C. pictus* and *C. speciosus* showing inflorescence and extra floral nectary; F & G: Floral parts of *C. pictus* and *C. speciosus*; H & I: Stamens of *C. pictus* and *C. speciosus*; J: Carpel of *C. pictus*; K: Carpel of *C. speciosus* showing style embedded between two thecae of fertile stamen; L: Stigma & Style of *C. pictus*; M: Carpel of *C. speciosus* with calyx; N & O: T.S. of ovary of *C. pictus* and *C. speciosus*; P & Q: Floral visitors on *C. pictus* and *C. speciosus*.
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