

## ***In Vitro* Propagation of Medicinal Plant *Orthosiphun Stamineus* (Misai Kucing) Through Axillary Branching and Callus Culture**

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**Abstract:** *Orthosiphun stamineus* is a herbaceous plant that is popularly known as Misai Kucing. It is widely used in traditional medicine as diuretic agent. This study was divided into two parts that was the *in vitro* production of complete plantlet through axillary branching and callus culture derived from leaf explant. In axillary branching method, sterilization was conducted using 0.02mg/100ml of mercuric chloride followed by rinsing with 20% and 50% of Clorox for 20 minutes and 5 minutes respectively. This sterilization method was able to remove the contaminants from the surface of the axillary stem and almost 70% of the explants were survived. Axillary bud was placed on Murashige and Skoog (MS) basic medium and cultured for 1 month. The *in vitro* shoot was inoculated on MS medium which was supplemented with different concentrations of BAP and NAA. The medium that contained 1.0mg/L of BAP gave the best shoot multiplication (13.25) and shoot length (6.23cm) after 8 weeks in culture. Root formation in term of percentage of root (70%) and the number of root produced (10.50) were the best when shoot inserted into medium contained 6mg/L IBA after 3 weeks in culture. However, MS medium that was supplemented with 2 mg/L IBA enhanced in the root length (3.85 cm). Meanwhile, in callus culture, the leaf explant was placed on MS medium containing with various concentrations of 2,4-D for induction of callus. The optimum level of callus induction and proliferation rate (0.42) were obtained with 4mg/L 2,4-D. The callus cells were tested in medium with Evan's Blue staining and the result showed that the cells were embryogenic. However, the shoot induction from the callus was failed in all tested mediums containing different combinations of BAP and 2,4-D.

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### **1. Introduction**

*Orthosiphun stamineus* is one of the most valuable medicinal plants which provide rich medicinal compounds. It has been object of interest among researchers since early 20<sup>th</sup> century (Khamsah et al., 2000). *Orthosiphun stamineus* is distributed from India through Malaysia to tropical Australia, South East Asia, Soviet Union and Cuba and in early 1920 to Europe. It is also called as *Orthosiphun aristalus* Benth with common names of Java tea (Indubala, 2000), kidney tea or bladder tea. It also has local or vernacular names including Misai Kucing or Kumis Kucing.

#### **1.1. Plant Taxonomy and Morphology**

*Orthosiphun stamineus* is a perennial herb which can grow to the height of 150 cm (Ahamed Basheer and Abdul Majid, 2010) with quadrangular, poorly ramified and ascending stem. The leaves are regulated in opposite pairs. They are glabrous, simple, green, and with a lanceolate leaf blade and a serrate margin. The leaf apice is acuminate with an acute leaf base (Ahamed Basheer and Abdul Majid, 2010). The petiole is partially short about 0.5 to 2cm in length with cuneate at base, acute or acuminate at leaf apical. The stem is quadrangle, reddish in color, and erect

with profuse branching. Flowers are borne on verticals about 16cm in length and have campanulate shape. They are white to bluish in color with long exerted filaments that make the flowers look cat's whiskers. Bracts are green, minute (1-2mm) and caudiform in shape. In nature, the flowers are hermaphrodite, about 6.2cm in length (including the stamen). There are two calyx lobes which are greenish red in color, having about 6 mm length and partially gamosepalous. One of the calyx margins is toothed and the other one is entire, both covered with white hairs. There are also two corolla lobes that are partially gamopetalous and covered with minute hairs. The corolla is light violet in color with lobes that are much shorter than the corolla tube. The corollas are bilabiate in shape with fringed margin. The labellum is light violet in color, hairy and pinkish on the under surface. There are 4 stamens which are inserted near the base of the corolla tube. There is a single, central, terete style with a clavate stigma. The fruit splits into 4 oblong-ovoid nutlets with 1.5 to 2.0 mm long.

#### **1.1. Growth Condition**

*Orthosiphun stamineus* can grow well in wet soil and be found in both temperate and tropical garden (Hsuan, 1986). In traditional breeding and