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Phytochemical quantification in various plant parts of *Aphanamixis polystachya* (Wall.) parker

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Abstract
Aphanamixis polystachya (Wall.) Parker is an evergreen medicinal tree in the Meliaceae family showed high therapeutical potential in traditional and modern medicines. Terpenoids were the major class of compounds found in this plant, and the majority of these compounds have high anticancer activity. Bark was considered as the primary source of isolation of these secondary metabolites. The present study was used for the identification of an equally important plant part from *A. polystachya* for the isolation of terpenoids. Apart from this; secondary metabolites like alkaloids and flavonoids were also quantified in various parts using UV-Vis spectrophotometry. It was observed that a higher concentration of terpenoids in leaves, whereas alkaloids were seen in high quantity in fruits and flavonoid was seen in higher quantity in the bark. Based on the result, leaves from *A. polystachya* can be used for the isolation of terpenoids.

Keywords: *Aphanamixis polystachya*, terpenoid, quantification, piscidinol

Introduction
Aphanamixis polystachya (Wall.) Parker is an evergreen tree native to India and was distributed in South-East Asian countries. *A. polystachya* has various medicinal properties and is used against various diseases by traditional healers such as liver disorders, rheumatism, tumors, and ulcers [1]. The research conducted in this species reported the high antioxidant activity [2], anti-inflammatory activity [3], hepatoprotective activity [4], and anticancer activity [5]. Based on these properties compound isolations were carried out and variety of compounds were reported from this plant such as aphanamixin [6], aphapolin A & B [7], aphagranol[8], polystanin [9], aphanalide, nemordisin, meliasenin, and agladupols [10], aphanamene, rohithukine [11], amooranin [12], and aphanin [13]. All these isolated compounds from *A. polystachya* exhibited various activities like antimicrobial, antioxidant, anti-inflammatory, and anticancer properties. In *A. polystachya*, majority of these compounds were isolated from the bark, and it was leading to the destruction of plants in the wild. The present study envisages determining of phytochemical quantification in different parts of the plant to find out alternate candidates for secondary metabolite isolation.

Materials and methods
Collection of plant materials.
The stem (bark), leaves, flowers, young & mature fruits were collected from ~ 50-year-old *Aphanmixis polystachya* tree from the home garden in Kerala, Parassala (8.3478^0 N and 77.141^0 E). The seeds were collected from the tree and seedlings were maintained in the glass house, Department of Botany, University of Kerala. For phytochemical analysis, along with the plant parts collected from the tree, stem, and leaves of 2-month-old seedlings (juvenile plants) were also used as source materials.

Phytochemical analysis
The phytochemical characterization was carried out on stem and leaf of juvenile plants along with stem (bark) and leaves of the mature tree, flower, immature fruits, and mature fruits (Fig.1). Extracts taken from fresh plant materials were quantified using UV visible spectroscopy

Quantification of Alkaloid [14]
Total alkaloid content was estimated using the Bromocresol green method, and atropine was used as a standard. Atropine was prepared by dissolving 1 mg in 10 ml distilled water. One gram of plant tissue was frozen in liquid nitrogen and grounded in a mortar. The powdered plant material was transferred to a graduated centrifuge tube and extracted with 10 ml methanol for 72 hours.

The extract was centrifuged at 4 ^{0}C to remove debris, and the supernatant was collected and dried. It was then dissolved in 1 ml of 2 N Hydrochloric acid and filtered. The non-polar fraction of filtrate was separated using 10 ml chloroform, and the step was repeated for three times. The P^H of the solution was neutralized using 0.1 N NaOH. To this solution, 5 ml of Bromocresol green reagent (69.8 mg Bromocresol green in 3 ml 2N NaOH and 5 ml distilled water; diluted to 1 liter in distilled water) and 5 ml phosphate buffer (2M sodium phosphate with 0.2 M Citric acid, P^H 4.7) were added. The mixture was shaken, and the complex was extracted with 1ml, 2ml, 3 ml, and 4 ml of chloroform by vigorous shaking. The non-polar fractions were collected in 10 ml volumetric flask and diluted with chloroform and absorbance was read at 470 nm.

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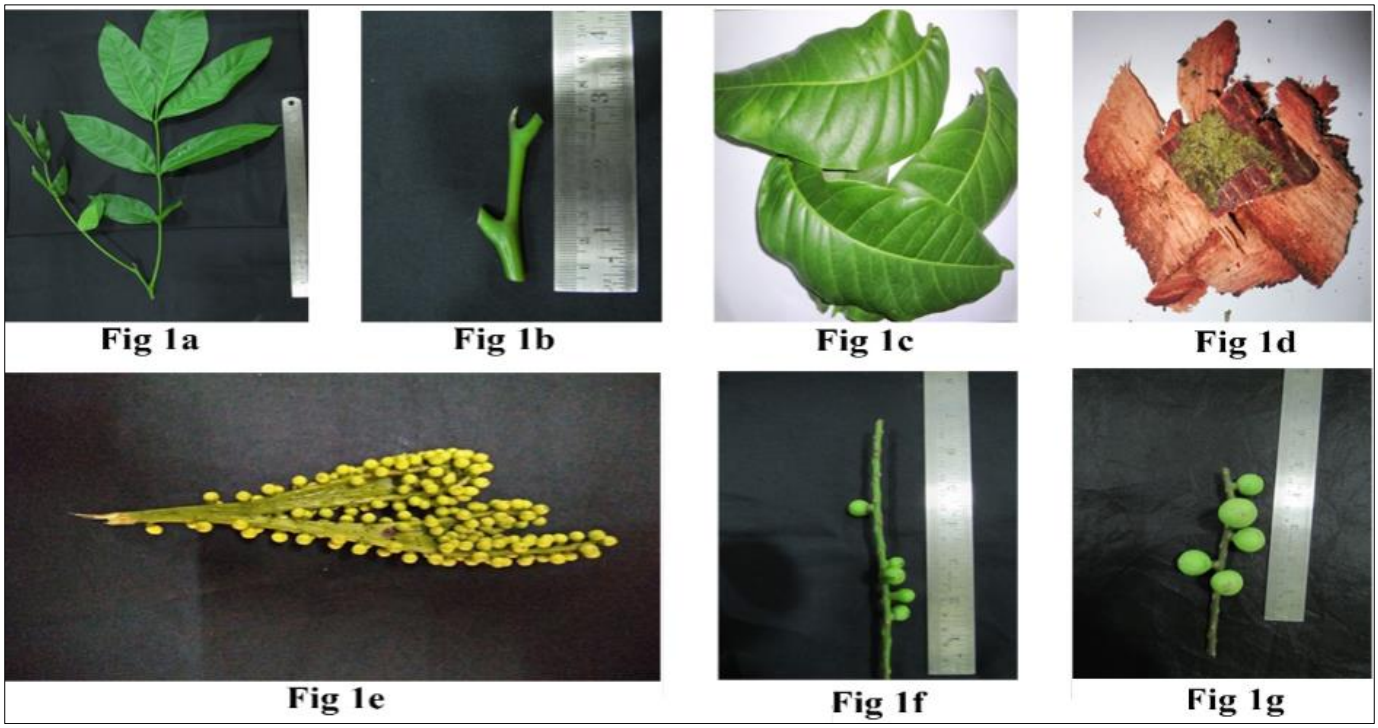

Fig 1: Plant parts used for quantification of secondary metabolites. a, leaves of young plants (Leaf Juvenile) b, stem of young plants (Stem Juvenile) c, leaves of tree (Leaf Mature) d, bark of tree (Stem Mature) e, Flower f, Immature Fruit g, Mature Fruit

Quantification of Flavonoids [15]

The plant tissue (100 mg) was grounded in liquid nitrogen and extracted in 1 ml methanol on the Eppendorf tube. The residue was centrifuged, and the filtrate was taken for analysis. To this extract 300µL of 5% sodium nitrate ($NaNO_2$) was added, followed by 300µL of 5% Aluminum chloride ($AlCl_3$) and incubated at room temperature for 5 minutes. After incubation 2 ml 1N Sodium Hydroxide (NaOH) was added; immediately, the volume of the solution was adjusted to 10 ml using distilled water. The solution was mixed properly, and the absorbance was determined at 510nm. A calibration curve was made by standard Rutin and estimated total flavonoid content in plant samples.

Quantification of Terpenoids [16]

The plant tissue (100mg) was grounded in liquid nitrogen. The powdered sample in the Eppendorf tube was extracted with 1ml methanol for 48 hours in the dark and centrifuged for 15 minutes at 12000 rpm to remove debris. The 200µL of supernatant was added to 1.5ml chloroform in 2 ml Eppendorf tube, mixed using vortex cyclomixer and incubated at room temperature for 3 minutes. After incubation, 100µL concentrated. Sulphuric acid (H_2SO_4) was added and incubated at room temperature for 2 hours in the dark. At the end of incubation reddish brown precipitate was formed in each Eppendorf tube. The precipitate was separated from the supernatant, and 1.5ml methanol was added to the precipitate. After mixing thoroughly, absorbance was analyzed in UV vis spectrophotometer at 538 nm. The OD values are plotted in a standard graph. Piscidinol was used as the standard for plotting graph.

Result and Discussion

Quantification of Alkaloids

Alkaloids were quantified by UV-Visible spectroscopy in methanolic extract of different plant parts of *A. polystachya*. The total alkaloid concentration was quantified in micrograms. Atropine was used as standard (Fig. 2a). Alkaloid content was maximum in mature fruits (671.11 ± 51.57 µg/gm) followed by flowers (547 ± 27.09 µg/gm) and leaves of juvenile plants(516.44 ± 26.28 µg/gm). Leaves of mature plants (496 ± 16.96 µg/gm) have high alkaloid content than that of immature fruits (442.67 ± 21.8 µg/gm). The stem of the juvenile plant (388.44 ± 9.49 µg/gm) showed more alkaloid than that of the stem of the mature plant (368 ± 12.13 µg/gm) (Fig 2b).

The total alkaloid concentration was similar in all plant parts whereas, flavonoids and terpenoids were minimum in the seedlings. In *Pinus ponderosa*, significant variation in foliar alkaloids was observed on the seedlings due to environmental and genetic changes [17]. Regional variation exceeded variation among families indicating a genetic regulation of alkaloid biosynthesis. However, the present study, since a single tree was used as source material, little variation was expected among different plant parts due to environmental factors. Alkaloid production and abundance were influenced by factors that affect the growth of fresh plant tissues such as light, soil nutrients, moisture and temperature [18] and

maintenance of seedlings in the uniform environment also reduced the variation in alkaloid content. It was observed that alkaloids show larvicidal properties against moth larvae [19]. The highest concentration of alkaloid was observed in fruits of *A. polystachya*, which indicates protection of fruits and seeds from predatory.

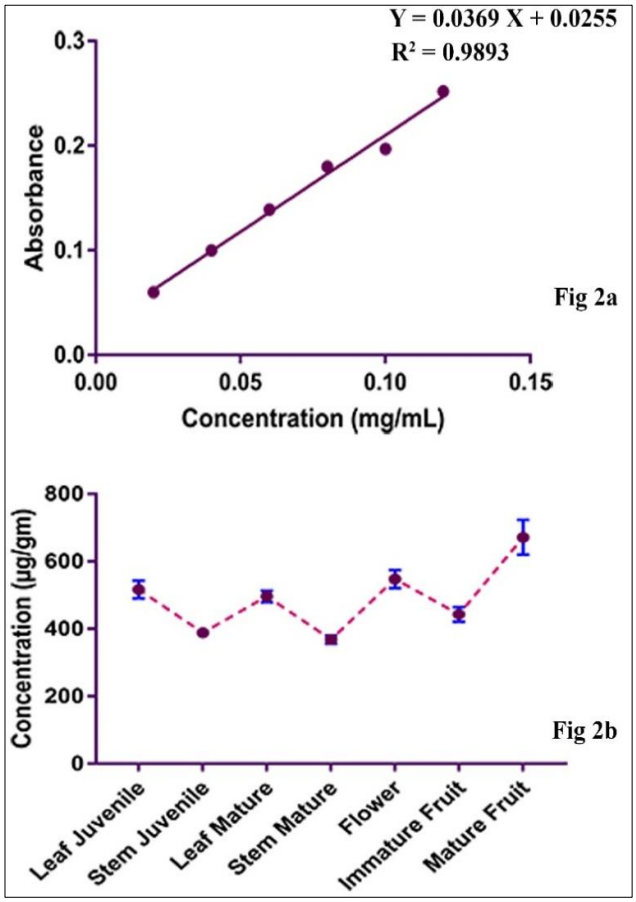

Fig 2: Quantification of alkaloid on different plant parts. a, The absorbance of varying concentration of standard Atropine. b, Total alkaloid concentration of different plant parts in A. polystachya.

Quantification of Flavonoids

Total flavonoid content of *A. polystachya* was quantified at milligram. Rutin was used as standard (Fig.3a). The maximum concentration was found in the stem of the mature plant (2.08 ± 0.2 mg/gm). The lowest concentration was found in the leaves of juvenile plants (0.59 ± 0.03 mg/gm). The immature fruit (1.24 ± 0.09 mg/gm) and mature fruit (1.29 ± 0.15 mg/gm) have an approximately equal concentration of flavonoids. The flavonoid content of the mature leaf was less than that of fruits with a concentration of 1.15 ± 0.27 mg/gm. The stem of the juvenile plant (0.76 ± 0.04mg/gm) has more flavonoids than the flowers (0.66 ± 0.1 mg/gm) (Fig.3b).

Flavonoids were common secondary metabolite in angiosperms, which confer a variety of biological functions in response with various stresses such as UV radiation, cold, heat, drought, salinity, herbivory, and pathogens, etc. [20]. Hence individuals in different populations exposed to varying environmental conditions usually showed variable accumulation of flavonoids [21]. In *A. polystachya*, the concentration of flavonoid was observed to be high in bark while leaves and other parts showed the approximately equal quantity of flavonoids indicating that those parts can be used as an alternative source for the isolation of flavonoids.

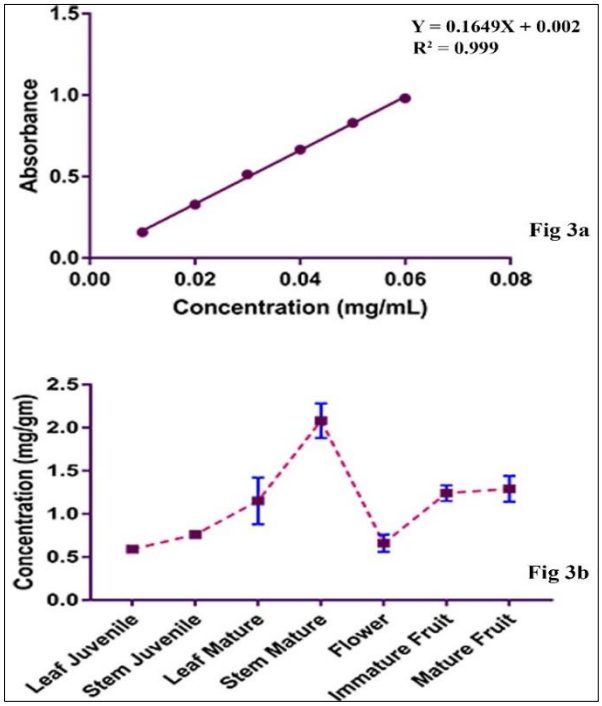

Fig 3: Quantification of flavonoids on different plant parts. a, The absorbance of varying concentration of standard Rutin. b, Total flavonoid concentration of different plant parts in A. polystachya.

Quantification of Terpenoids

Total terpenoid content of *A. polystachya* was quantified at milligram. Piscidinol was used as standard (Fig. 4a). Maximum terpenoid was observed in the leaves of mature plants (13.11 ± 0.36 mg/gm) followed by the stem of the mature plants (8.55 ± 0.51 mg/gm). Flower (7.1 ± 0.9 mg/gm) and mature fruit (7.26 ± 0.6 mg/gm) showed significant amount of terpenoids than immature fruits (5.96 ± 1.06 mg/gm). Least quantity of terpenoid was found in leaves of juvenile plants (1.54 ± 0.3 mg/gm), and the higher amount was observed in the stem of juvenile plants (2.91 ± 0.21 mg/gm) (Fig. 4b).

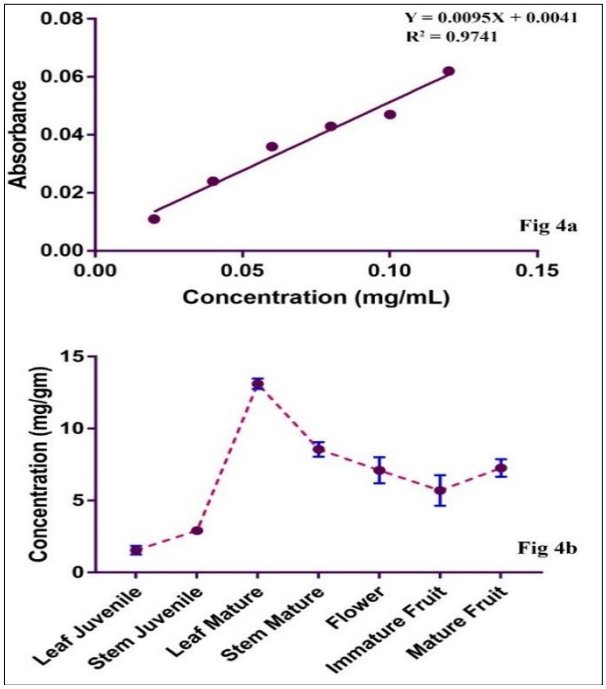

Fig 4: Quantification of terpenoids on different plant parts. a, The absorbance of varying concentration of standard Piscidinol. b, Total terpenoid concentration of different plant parts in A. polystachya.

Terpenoids have been identified as toxic or may repel herbivore attack and indirectly confer plant defense. There were changes in the proportion of specific terpenoids at different locations within a tree, resulting from the accumulation, transformation, and emission of these terpenoids. Majority of important phytochemicals isolated from bark, leaves, and roots in *A. polystachya* were seen in all organs except in fruits. Many compounds isolated from fruits was unique to mature or young fruits [9]. The concentration of terpenoids was found to be high in leaves compared to other organs. From this leaves can be considered as an alternate source for the production of terpenoids.

Conclusion

Terpenoids were the major class of phytochemicals isolated from *A. polystachya*, and it showed many therapeutical properties. In the present study, it was observed that similar to terpenoids; flavonoids were also seen in higher concentration. Majority of terpenoids were isolated from the bark of the plant, and it was observed that leaves could also be used as a major source of isolation of terpenoids. Similarly, it was observed that young plants showed less quantity of terpenoids, alkaloids, and flavonoids and all other parts in the mature plant can be used for the isolation of secondary metabolites

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