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# Quantitative Analysis of Selected Secondary Metabolites in the Leaves and Flowers of *Pogostemon Quadrifolius* (Benth.) F. Muell. (Lamiaceae)

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**Abstract** : *Pogostemon quadrifolius*(Benth.) is shrub used in folk medicine and it shows mosquito larvicidal, antimicrobial, anti cancerous and anti oxidant properties. The present study deals with the quantitative estimation of selected secondary metabolites; total alkaloids, saponin and tannin present in the leaves and flowers of the plant. The total alkaloid and saponin content is more in leaves ( $38.60 \pm 0.58 \text{ mg/ g}$ ,  $20.70 \pm 1.25 \text{ mg/g}$ ) than flowers respectively. Tannin content is found high in the methanolic extracts of leaves ( $21.67 \pm 2.65 \text{ mg GAE/gm}$ ) and flowers ( $16.59 \pm 1.51 \text{ mg GAE/gm}$ ).

Key Words : Pogostemon quadrifolius, alkaloids, saponin, tannin, leaves, flowers.

## Introduction

In plants, in addition to carbohydrates, proteins and fats, there are a wide range of phytochemicals called secondary metabolites which are used as pharmaceuticals, agrochemicals, flavours, fragrances, colours, biopesticides and food additives. The number of known chemical structures is estimated to be nearly fourfold greater than that in the microbial kingdom. The United State market sales of plant medicinals have risen up about US\$ 3 billion per year <sup>1</sup>.Secondary plant metabolites have biological properties such as antioxidant activity, antimicrobial effect, modulation of detoxification enzymes, stimulation of the immune system, decrease of platelet aggregation and modulation of hormonemetabolism and anticancer property. There are more than thousand known and many unknown phytochemicals. It is well-known that plants produce these chemicals to protect themselves, but recent researches demonstrate that many phytochemicals can also protect humans against diseases <sup>2</sup>.

Tannins are an important group of secondary metabolites and are defined as phenolic compounds of high molecular weight ranging from 500 Da to more than 3000 Da which are found in plants leaves, bark, fruit, wood and roots located basically in the tissues in the vacuoles. They have been closely associated with plant defense mechanisms against mammalian herbivores, birds and insects <sup>3, 4</sup>. Several health benefits have been recognized for the intake of tannins and some epidemiological associations with the decreased frequency of chronic diseases have been established <sup>5</sup>.In medicine, especially in Asian (Japanese and Chinese) natural healing, the tannin-containing plant extracts are used as astringents, against diarrhoea, as diuretics, against stomach and duodenal tumours<sup>6</sup>, and as antiinflammatory, antiseptic, antioxidant and haemostatic pharmaceuticals<sup>7</sup>.

Alkaloids are natural products that contains heterocyclic nitrogen atoms and are basic in character. Alkaloids are significant for the protection and survival of plants because they ensure their survival against micro-organisms (antibacterial and antifungal activities), insects and herbivores (feeding deterrens) and also againstother plants by means of allelopathically activechemicals<sup>8</sup>. Alkaloids have many pharmacological activities including antihypertensive effects ( many indole alkaloids), antiarrhythmic effect (quinidine,spareien), antimalarial activity (quinine), and anticancer actions (dimeric indoles, vincristine, vinblastine)<sup>9</sup>.

Saponins are a group of secondary metabolites found widely distributed in the plant kingdom. They form a stable foam in aqueous solutions such as soap, hence the name "saponin"<sup>10</sup>.Manysaponins are known to be antimicrobial, to inhibit mould, and to protect plants from insect attack. Saponins may be considered a part of plants' defence systems, and as such have been included in a large group of protective molecules found inplants named phytoanticipins or phytoprotectants <sup>11</sup>.These structurally diverse compounds have alsobeen observed to kill protozoans and molluscs, tobe antioxidants, to impair the digestion of protein and the uptake of vitamins and minerals in the gut, to cause hypoglycaemia, and to act as antifungal and antiviral<sup>12,13,14,15</sup>.

*Pogostemon quadrifolius* (Benth.) is a shrub distributed in India, Bangladesh and Myanmar<sup>16,17</sup>. The plant is used as folk medicine in India and Bangladesh for the treatment against chicken pox worms and also as a blood purifier<sup>18,19,20,21,22</sup>. The leaf extracts of *P. quadrifolius* (Benth.) exhibited antiproliferative property due to the presence of a new compound (Z)-ethylidene-4,6-dimethoxycoumaran-3-one and it induces apoptosis in cancer cell line<sup>23,24,25</sup>. *P. quadrifolius*(Benth.) leaf methanol extract showed DPPH radical scavenging property due to the presence of phenolic compounds <sup>26</sup>. The plant also exhibits mosquito larvicidal and antimicrobial property<sup>27,28,29,30</sup>. The preliminary phytochemical screening and GC MS analysis of various extracts of leaves and flowers of *P. quadrifolius* (Benth.) has shown the presence of various compounds like alkaloids, phenols, flavonoids, terpenes, tannin, saponin etc., in various extracts and the flavonoid and phenolic contents of leaves and flowers has been studied by spectrophotometric method <sup>31,32</sup>. In order to explore the possible medicinal potential, the present study is aimed to determine the total alkaloids, tannin and saponin contents of the leaves and flowers of *Pogostemon quadrifolius* (Benth.) (Lamiaceae).

#### **Materials And Methods**

#### **Collection of plant materials and extraction**

*Pogostemon quadrifolius* (Benth.) was collected from Panakkad, Karimbam, Taliparamba, Kannur and authenticated by Dr. A.K.Pradeep, Department of Botany, University of Calicut. The leaves and flowers were washed thoroughly and dried in shade. The dried leaves and flowers were then powdered using a mixer grinder and the powder was kept in small airtight bottles with proper labeling. The powdered leaves and flowers were extracted sequentially using petroleum ether, acetone and methanol in increasing order of polarity using soxhlet apparatus until all the constituents were completely eluted. The extracts were then filtered and evaporated to dry. The dried solvent extracts were used for further studies. Powdered leaves and flowers were used in quantitative estimation of alkaloids and saponin.

#### **Determination of total tannins**

Tannin content in each extract was determined using insoluble polyvinyl-polypirrolidone (PVPP), which binds tannins as per standard procedures  $^{33,34,35}$ . Briefly, 1 ml of extract dissolved in methanol (1 mg/ml), in which the total phenolics were determined, was mixed with 100 mg PVPP, vortexed, kept for 15 min at 4°C and then centrifuged for 10 min at 3,000 rpm. In the clear supernatant the non-tannin phenolics were determined the same way as the total phenolics<sup>36,37,38,39</sup>. Tannin content was calculated as a difference between total phenolic and non-tannin phenolic content.

#### **Determination of total alkaloids**

5g of the sample was taken into a 250ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 hrs. This was filtered and the extract was concentrated on a water bath to one – quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed <sup>40</sup>. The samples were ground and 20 g each were put into a conical flask and 100 cm3 of 20% aqueous ethanol were added. The samples were heated over a hot water bath for 4 hrs with continuous stirring at about 55 C. The mixture was filtered and the residue re extracted with another 200 ml 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90 C. The concentrate was transferred into a 250 ml separatory funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated , 60 ml of n – butanol was added. The combined n – butanol extracts were washed twice with 10 ml of 5 % aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in the oven to a constant weight ; the saponin content was calculated <sup>41</sup>.

#### **Results and Discussion**

**Determination of total saponins** 

The total alkaloid and saponin content in the leaves and flowers of *P. quadrifolius* is provided in table 1. The total alkaloid and saponin content is more in leaves  $(38.60 \pm 0.58 \text{ mg/ g}, 20.70 \pm 1.25 \text{ mg/g})$  than flowers respectively. Non tannin and tannin content of leaves and flowers of various extracts of *P. quadrifolius* has been provided in table 2 &3 along with total phenolic content <sup>32</sup> for comparison. Tannin content is found high in the methanolic extracts of leaves  $(21.67 \pm 2.65 \text{ mg GAE/gm})$  and flowers  $(16.59 \pm 1.51 \text{ mg GAE/gm})$  (Figure 1 &2). The study suggests that the plant contains significantly high amount of alkaloids, saponin and tannin and moreover leaves contain more amount of secondary metabolites than flowers. The study will lead to further research for isolating, identifying and structural elucidation of bioactive components by modern techniques.

Name of secondary metabolite	Leaf	Flower		
Alkaloid	$38.60 \pm 0.58$ mg/ g	$35.17 \pm 0.85 \text{ mg/g}$		
Saponin	$20.70 \pm 1.25 \text{ mg/g}$	$17.17 \pm 1.03 \text{ mg/g}$		

Table 1. The total alkaloid and saponin content present in the leaves and flowers of *P. quadrifolius* 

Values are expressed as mean  $\pm$  SD of three replicates.

Table 2. T	he total	phenolic,	tannin	and	non	- tannin	content	present	in	the	various	leaf	extracts	of <i>P</i> .
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Parameter	Unit	Petroleum ether extract	Acetone extract	Methanol extract
Total	mg GAE/gm	$17.06 \pm 0.69$	$138.57 \pm 3.10$	163.57 ±3.10
phenolic <sup>32</sup>				
Non tannin	mg GAE/gm	$12.62 \pm 1.03$	$119.05 \pm 2.38$	$141.90 \pm 3.43$
Tannin	mg GAE/gm	$4.44 \pm 1.55$	$19.52\pm3.98$	$21.67\pm2.65$

Values are expressed as mean ± SD of three replicates. mg GAE/gm(milligram Gallic acid equivalent/gm)

# Table 3. The total phenolic, tannin and non - tannin content present in the various flower extracts of *P*. *quadrifolius*

Parameter	Unit	Petroleum ether extract	Acetone extract	Methanol extract
Total phenolics <sup>32</sup>	mg GAE/gm	$5.24\pm0.63$	$76.27 \pm 1.20$	$147.06\pm1.53$
Non tannin	mg GAE/gm	$3.05 \pm 1.07$	$62.14 \pm 0.63$	$130.48 \pm 0.24$
Tannin	mg GAE/gm	$2.19\pm0.46$	$14.13 \pm 1.31$	$16.59 \pm 1.51$

Values are expressed as mean  $\pm$  SD of three replicates.



Figure 1. Tannin content in the various extracts of leaves of P. quadrifolius



Figure 2. Tannin content in the various extracts of flowers of *P. quadrifolius* 

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