Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2017; 6(6): 1942-1945
Received: 19-09-2017
Accepted: 20-10-2017

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# Phytochemical analysis of secondary metabolites on *Pogostemon auricularis* (L.) Hassk. and *Anisomeles malabarica* (L.) R. BR. ex Sims

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**Abstract**
The present study was made an attempt to compare the preliminary phytochemical constituents of the leaf extracts of two extreme environments such as: hydrophytic (*Pogostemon auricularis*) and xeric (*Anisomeles malabarica*) plants. The dried leaf sample was extracted with three different solvents such as petroleum ether, methanol and water. The quantitative analysis of the total phenolics, flavonoids, tannin and alkaloids were assayed using the standard protocols. The maximum yield of extract (28% W/W) was record in *P. auricularis* of methanol extract. But, the *A. malabarica* showed maximum (20% W/W) extraction in methanol extract. Of the different solvent extractions the highest concentration secondary metabolite was identified with the total phenolic content in methanol extract of *P. auricularis* (54.59±0.5 (mg GAE/g extract) and *A. malabarica* of (48.28±0.5 mg GAE/g extract) respectively. The present investigation found that the remarkable variation in flavonoids, tannin and alkaloids. It may pave a new way to identify the novel secondary metabolites.

**Keywords:** Secondary metabolites, *Pogostemon auricularis*, *Anisomeles malabarica*, Phenolics, Alkaloids, Lamiaceae.

## 1. Introduction

The medicinal plants are the repository for bioactive compounds, which are exclusive source for phytodrugs and it has many selective advantages over the synthetic drugs. The bioactive compounds have been occurring in plants used for human health and it has been employed to cure a wide range of disease such as antiallergenics, antidiabetics, antioxidants, antimutagenics, anticarcinogenics, antimicrobial, and anti-inflammatory agents, enhancers of the gastrointestinal function, and immune-modulators [1]. Phytochemical are synthesized based on their requirement and need of the plants. However, it production have been influenced by the external factors and internal stimuli [2]. Indeed, the secondary metabolites are not directly involved in the normal growth and development of plants [3] has many functions like to protect from predators and microbial pathogens, to herbivores and UV-B radiation. [4].The metabolic turnover of the phytochemical compounds, have been intensively studied in higher plants. Interestingly, the leaf synthesized many secondary metabolites that are translocate to stem and stored in the root. For instance, the alkaloid nicotine have been synthesized in root and transported to leaves through three specific transporter of root cell, leaf cell and vacuole plasma membrane [5]. The secondary metabolites are transported both inter and intracellular mode and to be stored in specific parts organ and also in organelles. The recent research on molecular studies have been clearly identified the specific genes of synthesis; transportations and storage [5]. It has been synthesized in roots and to transport in leaves. On the base utilitarian the secondary metabolites are used for pharmaceuticals, food additives, flavors, and other industrial materials [6].

Among the angiospermic plants, Lamiaceae members are have been used in various use ethanomedicine and traditional medicinal system across the world for diuretic, sedative, digestive, antiparasitic, carminative, appetizer, anticonvulsant and antiiflammatory [7]. Indeed, most of the Lamiaceae member aromatic and predominantly endowed with phenolic compounds, essential oils and terpenoidal group [8, 9]. In India, 64 genera and 350 species were located and commonly in Mediterranean region [10]. Lamiaceae members are distributed and well thrive in all climatic zone. *Pogostemon auricularis* is one of the medicinal herb and identified as an endemic plants in India [11]. It's also used to cure for hysteria, diarrhoea, stomachache and discomfort [12]. *Anisomeles malabarica* is an another important Lamiaceae member commonly seen in dry place of plain region and considered as a vital herb to cure cancer, liver disorder, fever, cold and cough [13] and wound [14].

The main objective of the present study is, to quantify the secondary metabolites of total phenolics content, flavonoids, tannin and alkaloids on *P. auricularis* and *A. malabarica*

## Materials and Methods

*Pogostemon auricularis* (L.) Hassk. (Fig.1) was collected from Kolli hills, Namakkal Dist. and the population exclusively distributed along the streams around 1100 MSL (Mean Sea Leval). The *Anisomeles malabarica* (L.)R.Br.ex Sims (Fig.2) was collected from the range of land Kadavur hills, Karur Dist. Tamil nadu. The plant population was the dominant mostly in arid zone and 206 MSL. Both these plants were authenticated identified by Botanical Survey of India (BSI/ SRC/5/23/2015/Tech/1549) (BSI/ SRC/5/23/2015/Tech/1546), Coimbatore, Tamilnadu, India.

**Fig 1:** *Pogostemon auricularis*

**Fig 2:** *Anisomeles malabarica*

### Plant extract preparation

The plant materials were dried at room temperature for a week. The dried leaves were grinded by a mechanical grinder and passed through 40 micron mesh sieve. The extractives were extracted used by cold maceration method. The powder materials (50g) were taken in conical flask and different solvent (250ML) of Petroleum ether, Methanol and Hot water (boil $60^0$C for 45 min) for 24 hours and then filter through whatmann No.1 paper. The extract were collected and evaporated by rotary vaccum evaporated and stored at $4^0$C in refrigerator for further use.

### Estimation of total phenolics, flavonoids, tannin and alkaloid content.

The total phenolic content was determined according to the method described by [15] method. The results were express as gallic acids equivalent (GAE)/g extract. The flavonoids content of sample was determined by the use of a slightly modified colorimetric method described by [16]. Rutin used as a standard value. The tannin contents were analysed after the treated with polyvinyl polypyrrolidone (PVPP) [17]. The tannin content of the sample was calculated by subtracting the non-tannin phenolics from total phenolics. The alkaloids content of all the plant extracts were determined following to the method described by [18]. All the experiments were done in triplicate.

## Results and Discussion

The present work on the quantification of phytochemical compounds was carried out in leaf samples of both *P. auricularis* and *A. malabarica*. As mentioned in materials and methods, the dried leaves samples were dissolved petroleum ether, methanol and hot water. For the phytochemical extraction and estimation are a wide range of solvent have been used to obtain the available bioactive compound in the sample. Maceration methods of extraction is best and easiest technique when compare to other themolabile technique. The soluble phytochemical compounds were easily dissolved in solvent [19]. The highest yield of 28% W/W extract was record in *P. auricularis* of methanol extract, followed by Hot water (14 % w/w) and the lowest extract percentage was found in petroleum ether 5.23(% W/W). Similarly in *A. malabarica* leaf methanol extract record as a maximum of 20% W/W and lowest percentage (8 % w/w) was recorded in petroleum ether. However, *A. malabarica* leaf hot water extract yield 12.23%. The present work was found the maximum amount of phytochemical compounds obtained in methanolic extract of both plant samples shown table.1.

**Table 1:** Quantitative estimation of total phenolics, flavonoids, tannin and alkaloid content by using different solvents in the leaf samples of *P. auricularis* and *A. malabarica.*

| Name of the plants | Phytochemical compounds | Petroleum ether | Methanol | Hot water |
|---|---|---|---|---|
| *P. auricularis* | Total phenolics (mg GAE/g extract) | 12.34±0.2 | 54.59±0.5 | 35.72±0.1 |
| | Flavonoids (mg RE/g extract) | 9.26±0.2 | 46±1 | 32.57±0.3 |
| | Tannin (mg GAE/g extract) | ND | 32.57±0.3 | 13.73±0.2 |
| | Alkaloids (mg RE/g extract) | ND | 12.62±0.1 | 8.38±0.1 |
| *A. malabarica* | Total phenolics (mg GAE/g extract) | 14.41±0.3 | 48.28±0.5 | 28.15±0.5 |
| | Flavonoids (mg RE/g extract) | 12.39±0.1 | 28.26±0.5 | 23.56±0.2 |
| | Tannin (mg GAE/g extract) | 7.34±0.4 | 20±1 | 18.24± |
| | Alkaloids (mg RE/g extract) | ND | 16.48 ±0.4 | 0.88±0.9 |

**ND-** Not Detected.

Values are expressed by mean±SD of three samples, SD: Standard deviation

Similarly results were noted at *Achyranthes aspera* Linn. [20], *Leucaena leucocephala* [21]. The highest amount of total phenolic content were recorded in *P. auricularis* and *A. malabarica* of methanol (54.59±0.5 (mg GAE/g extract) (48.28±0.5 mg GAE/g extract), respectively. The lowest content was measured at *P. auricularis* and *A. malabarica* in petroleum ether extract (12.34±0.2 mg GAE/g extract) (14.41±0.3 mg GAE/g extract) respectively. Highly phenolic content was dissolved in polarity solvents, because high solubility of phenolic compounds showed in polar solvent extract [22], [23]. The similar results were reported in *Leucas linifolia* and *coleus aromaticas* (L) *Marrubium peregrium* [24]. The phenolic content is very important role of plant growth, development and disease resistance. Whereas, to protect from cancer, cardiovascular disease resistance, diabetes, Osteoporosis and neurodegenerative disease [25]. The highest content of flavonoids are measured at methanol extract (46±1 mg RE/g extract) and (28.26±0.5 mg RE/g extract) in *P. auricularis* and *A. malabarica*, followed by Hot water and petroleum ether. In angiospermic plants, the flavonoid compounds are synthesized and accumulate to induce by ultraviolet radiation, low temperature, and minor amount of nutrients, devoid of water and pathogen stress [26]. The flavonoids are based on polarity solvents because dissolved in methanol solvents [27]. Flavonoids are act as in different function in plants such as pigmentation and defense mechanism [28]. The highest content of tannin and alkaloids were showed in methanol extract and in *P. auricularis*, tannin (32.57±0.3 mg GAE/g extract) alkaloids (12.62±0.1 RE/g extract) showed highest content and *A. malabarica* leaf extract showed in tannin(20±1 mg GAE/g extract) alkaloids (16.48±0.1 mg RE/g extract). In *P. auricularis* plants are not detected in Tannin and alkaloid content in petroleum ether extract but *A. malabarica* have not detected in alkaloid content. Mainly, the tannin acts as a feeding repellent, to prevent spore germination and particularly the *Colletotrichum circinans* fungus [29]. Alkaloid is a largest family of N containing compounds and located in 20% of vascular plants [30]. Its act as protect from predators [31]. Similarly, [32], reported that the highest concentration of alkaloids content were recorded in *Ocimum santum, Hyptis suavelonse and Plectranthus mollis* in Lamiaceae members

## Conclusion

The synthesis, accumulation and storage of secondary metabolites in higher plants have been studied by researcher across the world. The biosynthesis of metabolites in plants have been reported by molecular studies, however, the turnover of the metabolites from primary category into secondary is highly special and temporal and it's depends on both internal and external factors. For instance, the leaves are primarily involved in phytosynthetic process, indeed many of the secondary metabolites are synthesized in leaves either by specialized cells or normal photosynthetic parenchymatic cells. The present study was estimated that the total phenolics, flavonoids, tannin and alkaloid in leaves of two Lamiaceae members i.e *P. auricularis* and *A. malabarica.* The Leaves of these two plants have been used to cure many ailments and an important source of medicinal plants in various traditional systems. The present phytochemical investigation remarkable showed variation in secondary metabolites by using solvents. In *P. auricularis* and *A. malabarica*, the methanol extract contained total phenolics, flavonoids, tannin and alkaloids with variable quantitative that was measured by mg/g.

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