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# Phytochemical, Antimicrobial and Antioxidant Activity of *Andrographis alata* (Vahl) Nees

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**Abstract:** ***This study has been undertaken to investigate the phytochemical, Antimicrobial and Antioxidant activity of Andrographis alata (Vahl) Nees. commonly known as Peria nangai (Tamil) which is used in Ayurveda to treat diseases like hepatitis, diabetes etc. in the present investigation solvents like, Petroleum ether, Methanol and Ethanol were used to extract the phytochemical content and in-vitro antimicrobial and antioxidant activity against certain bacterial species has been made. The results showed that the Methanolic extract had significant inhibiting activity of zone of inhibition. The antioxidant assay was done for the plant extract and free radical scavenging assay was made by measuring its capability for scavenging nitric oxide radicals, hydrogen peroxide radicals. Methanolic extract also showed highest effect in hydroxyl radical and nitric oxide scavenging assay.***

**Keywords:** *Andrographis alata*, Phytochemical, Antimicrobial, Antioxidant Activity

## 1. Introduction

Plants are important source of medicines and play a key role in world health. Almost all cultures from ancient times to today have used plants as medicines. Today medicinal plants are important to global economy. Approximately 85% of traditional medicine preparations involve the use of plants or their extracts. Medicinal plants have been used for centuries to cure human diseases [1, 2]. According to WHO the increase of resistance to antibiotics by bacterial pathogens is a growing problem in both developed and developing countries. It has been proved that many of these plants exhibit general antifungal and antibacterial activities. The systematic screening of antimicrobial efficacy by plant extracts represents continuous efforts to find newer compounds with the potential to act against multidrug resistance organisms [3]. The scientific investigations on the plant components to derive the efficacy of antimicrobial properties were first documented in the late 19$^{th}$ century [4].

*Andrographis alata* (Vahl) Nees. Belonging to the Family Acanthaceae commonly known as Peria nangai (Tamil) is a native to South India, Sri Lanka. It is an Undershrub, found growing in Moist deciduous forests, the formation of Flower and Fruit can be noticed in the month of November-March [5]. The medicinal shrub is used in the Indian systems of medicines such as Ayurvedha, Homeopathy, Naturopathy, Siddha and Unani [6]. *Andrographis alata* has been shown to possess antipyretic, anti-inflammatory, antivenom activity [7,8,9]. A new flavone 2'-glucoside and Acylated 5,7,2',6'-oxygenated flavone glycosides were isolated from the whole plant [ 10, 11, 12, 13].

## 2. Materials and Methods

### 2.1 Sample collection

Fresh parts (Whole plant) of *Andrographis alata* (Vahl.) Nees. was obtained from forests of Malebennur range, Davanagere and Joldhal range, Chitradurga, Karnataka, India. used as plant sample for the present investigation. The plant materials were washed thoroughly with running tap water and with distilled water to remove surface microflora and are shade dried, ground well into fine powder by using blender and stored in an airtight container for the use.

### 2.2 Preparation of Plant Extracts

#### 2.2.1 Aqueous extraction (Yadav & Agarwal, 2011 [24])

5gm of finely powdered plant materials of *Andrographis alata* (Vahl.) Nees. was taken in a beaker and 100ml of distilled water was added. The mixture was heated with continuous stirring at 30º-40ºC for 20 minutes. Then the water extract was filtered through filter paper and the filtrate was used for the present investigation.

#### 2.2.2 Solvent extraction

Crude plant extracts were prepared by using Soxhlet apparatus. About 50gm of powdered plant materials such as *Andrographis alata* Nees., was extracted with Petroleum ether, chloroform and methanol solvents respectively. The process of extraction was continued till the solvent in siphon tube of an extractor become colourless. Further, the extract evaporated completely in a small beaker and stored in refrigerator at 4ºC for the present investigation.

### 2.3 Preparation of test sample

The extracts were filtered, dried and 10mg of the dried sample was dissolved in 10ml of respective solvent (stock solution). 1ml of this solution was diluted to 10ml of respective solvent and the solution was further diluted to obtain 2-10µg/ml.

### 2.4 Phytochemical Screening:

Qualitative phytochemical analysis of the chloroform, methanol and distilled water extracts of *A. alata* Nees were carried out using standard procedures to identify the constituent alkaloids (Mayer's test), steroids and terpenoids (Liberman- Burchard and Salkowski tests), cardiac glycosides (Keller-Kilani test), saponins (foam tests), flavonoids (Shinoda test), tannins and phenols (Ferric chloride test) as described by [14,15,16] Sofowara (1993), Trease and Evans (1989) and Harborne (1993).

**2.5 Bacterial and Fungal Cultures**

**Procedure**
Different bacterial and fungal cultures were obtained from MTCC, Chandigarh. Bacterial cultures: *Bacillus cereus* (MTCC-2155), *Staphylococcus aureas.* (MTCC-3160) *Escherichia coli* (MTCC-443) and *Klebsiella pneumoniae* (MTCC-3384),

**Fungal cultures:** *Aspergillus niger* (MTCC- 9687)
The bacterial cultures were revived in nutrient broth medium and incubated at 37°C for 24 hours. Each bacterial culture was maintained at 37°C on nutrient agar slants and nutrient broth after every 48 hours of sub culturing. Fungal culture was revived on Potato dextrose agar by maintaining at 25°C for 5-7 days for further use.

**2.5 (I) Preparation of nutrient media:**

Beef extract 1.0 gm, yeast extract 2.0 gm, peptone 5 gm, sodium chloride 5.0gm and distilled water 1000 ml taken in a conical flask and the pH adjusted to 7.2 with pH meter 15 gm of agar was added and media was autoclaved at 121˚C for 20 minutes.

**2.6 Preparation of aqueous extract at various concentrations**

Powdered plant materials were used to prepare the aqueous extract. The aqueous extract was prepared at different concentrations (0.25g, 0.5g, 0.75g, 1.0g) with distilled water. The hot water extract of *Andrographis alata* (Vahl) Nees., was tested for the presence of bioactive compounds by using standard methods.

**2.7 Antimicrobial Activity - Agar well diffusion method:**

The antimicrobial activity of the Plant extracts was assayed separately using Agar well diffusion method in aseptic conditions. The disk diffusion method [ 17] by Kirby-Bauer, 1966 was adopted to assess the antimicrobial activity of prepared extracts. The standardized (10 µl- 100µl) bacterial and fungal stock suspension (CFU/ml) is used as inoculum. Sterile Whatmann No1 filter paper disks of 5 mm diameter were soaked into the seed extracts for a minimum of 2 hours and dried. Tetracycline disc is used as positive control. By using sterile forceps, seeded discs of the extract are placed onto each of the inoculated plates. Bacterial plates such as *Escherichia coli*, *Staphylococcus aureas*, *Bacillus cereus* and *Klebsiella pneumoniae*. are incubated at 37°C for 24 hours and fungal plates at 25°C for 5-7 days. After incubation antimicrobial activities is determined by measuring the diameters of zone of inhibition in mm. Each dose level is tried in triplicates.

**2.8 Nitric Oxide Scavenging Activity**

Nitric oxide radical scavenging activity was done according to the procedure of Garret 1964 [18] method and it was calculated according to the following formula

$$\% \text{ of inhibition} = \frac{(AO-A1) \times 100}{AO}$$

AO = absorbance of control, A1= Absorbance of extract

**2.9 Hydroxyl Radical Scavenging Activity:**

The Hydroxyl Scavenging activity was performed using Yu *et al.*, 2004 [19] procedure and the result was calculated by the following formula

$$\% \text{ of inhibition} = \frac{(AO-A1) \times 100}{AO}$$

AO = absorbance of control, A1= Absorbance of extract

## 3. Results and Discussion

The preliminary phytochemical analysis of *A. alata* (Vahl.) Nees revealed relative distribution of the secondary metabolite as shown in the Table 1 it also reveals that there is highest amount of presence of carbohydrates, phenols, alkaloids, flavonoids, tannins and Glycosides this is also in confirmation with the plant extracts of *A. paniculata* Nees. [6]. Alkaloids which are one of the largest groups of phytochemicals in plants have amazing effects on humans and this has led to the development of powerful pain killer medications [20, 21] revealed the inhibitory effect of saponins on inflamed cells. Steroidal compounds present in *A. alata* (Vahl) Nees extracts are of importance and interest due to their relationship with various anabolic hormones [7].

**Table 1:** Screening tests for secondary metabolites in solvents extracts of *A. alata* Nees

| Components | Tests | *Andrographis alata* Nees. | | |
|---|---|---|---|---|
| | | Chloroform | Methanol | Petroleum ether |
| Proteins | Millons Test | - | - | - |
| | Ninhydrin Test | - | - | - |
| Carbohydrates | Fehlings Test | + | + | + |
| | Benedicts Test | - | - | - |
| | Iodine Test | - | - | - |
| Phenols | Phenol test | + | + | + |
| Tannins | Tannin test | + | + | + |
| Flavonoids | Shinoda test | + | + | + |
| | Alkaline test | + | + | + |
| Saponin | Saponin Test | - | + | - |
| Glycosieds | Libermanns Test | - | + | - |
| | Salkowskis Test | - | - | + |
| | Keller-Kilani Test | - | + | + |
| Steroids | Control | - | - | + |

| | | | | |
|---|---|---|---|---|
| | Liberman-Burchard Test | + | - | - |
| Alkaloids | Mayers Test | - | + | - |

**Antibacterial activity:**

Antibacterial studies of the three different extracts (Petroleum ether, Chloroform and Methanol) of the *A. alata* (Vahl) Nees. Revealed that the Methanol extract had significant activity against *Klebsiella pneumonia* (50 µl -5mm; 40 µl-4.5mm and 30 µl-3.5mm), *Bacillus cereus* (50 µl-7mm; 40 µl-6.5mm and 30 µl-5.8mm) and *Escherichia coli* (50 µl-8mm; 40 7.5 30 µl-5.9mm) **Fig-2.** Similar observations are also made by D.S. Mohale, *et al.*, 2014 [22]. Chloroform extract showed significant activity against *Klebsiella pneumonia* (50 µl -7.3mm; 40 µl-6.2mm and 30 µl-5.3mm), *Bacillus aerus* (50 µl-5.2mm; 40 µl-4.6mm and 30 µl-4.0mm) and *Escherichia coli*. (50 µl-9mm; 40 µl 8.0mm 30 µl-6.8mm) (Fig 3). Petroleum ether extract showed significant activity against *Klebsiella pneumonia* (50 µl -3mm; 40 µl-2.8mm and 30 µl-1.7mm), *Bacillus aerus* (50 µl-2.8mm; 40 µl-2.2mm and 30 µl-1.8mm) and *Escherichia coli* (50 µl-5.2mm; 40 µl 4.6mm 30 µl-3.5mm) (Fig 1).

However Methanolic extract of *A.alata* showed activity against *Staphylolcoccus aureus* (50µl4mm; 40µl-2mm and 30µl-2mm) and did not show any activity against rest of the solvents extract (Fig 2). The results are similar to the previous investigators [10] worked on *A. paniculata* and [17].

**Zone of inhibition for bacterial cultures**

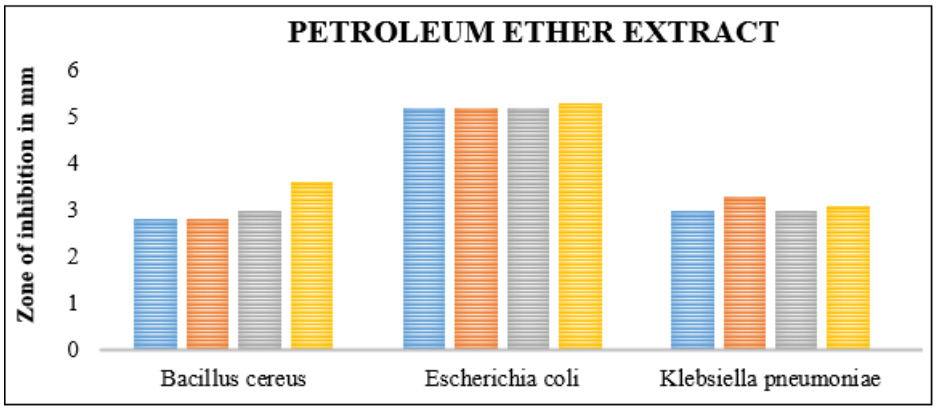



**Figure 1:** Graphical representation of inhibition observed in Petroleum ether extract

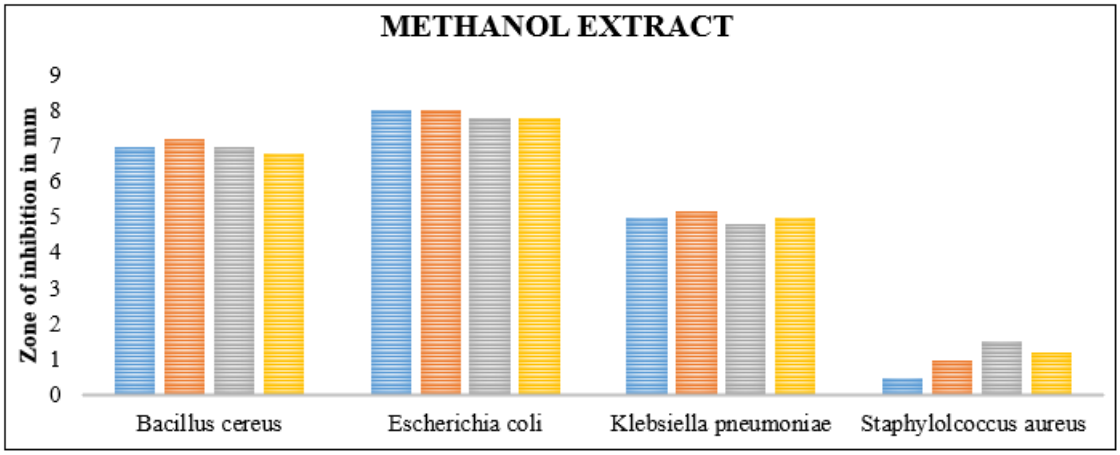



**Figure 2:** Graphical representation of inhibition observed in Methanol extract

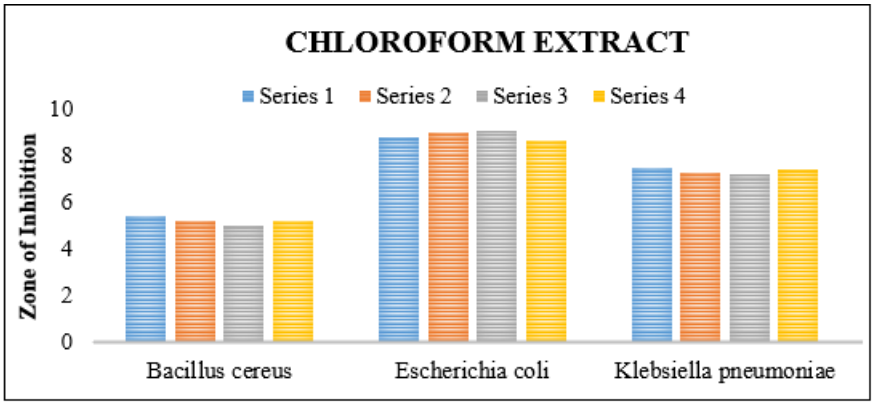
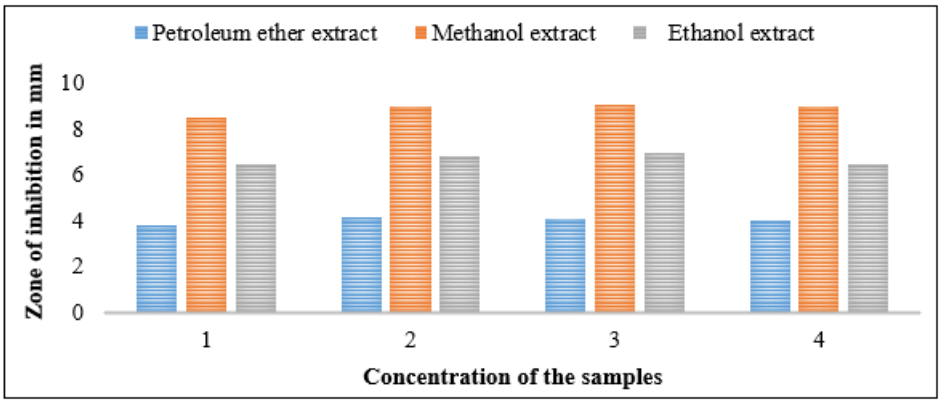



**Figure 3:** Graphical representation of inhibition observed in Chloroform extract

**Zone of inhibition for fungal cultures**

The zone of inhibition on *Aspergillus niger* was found to be more in methanolic extract at the concentration of 3mg/ml to 9.1 cm followed by Chloroform and Petroleum ether extract with the concentration of 3mg/ml to7cm. the results are concurrent with the report of by Hiradeve and Rangari 2015 [23] have made similar observations of antifungal activity of different extracts of *E. scaber* Linn. against different fungal strains from the aqueous and methanol extract of plant parts.




**Hydroxyl Radical Scavenging Activity**

The hydroxyl radical scavenging activity showed a dose independent manner. Percentage of inhibition was found in methanol extract at 4 µg/ml. The percentage of inhibition for *A. alata* was found to be highest at 4 µg/ml in methanol extract while no activity was found in chloroform extract (Fig 4).

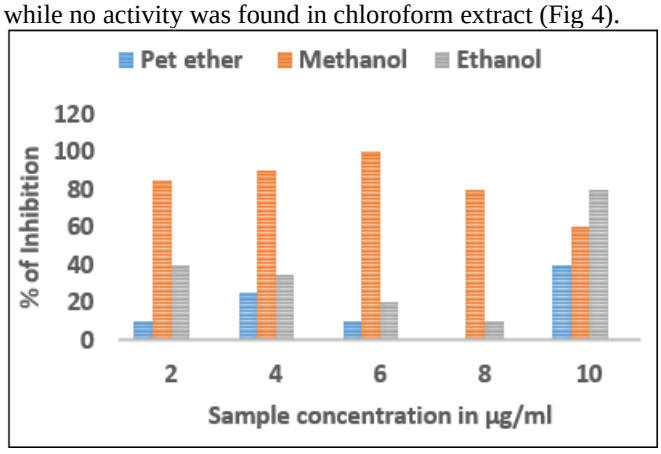



**Figure 4:** Graphical representation of % of inhibition observed in Hydroxyl Radical Scavenging Activity

**Nitric oxide Scavenging Activity**

The results of Nitric oxide scavenging activity exhibited in a dose independent manner. Percentage of inhibition was found in methanol extract at 6 µg/ml. The percentage of inhibition for A. alata was found to be highest at 6 µg/ml in methanol extract (Fig 5) this assay is based on the scavenging ability of the extracts. It was found to increase in dose dependent way.

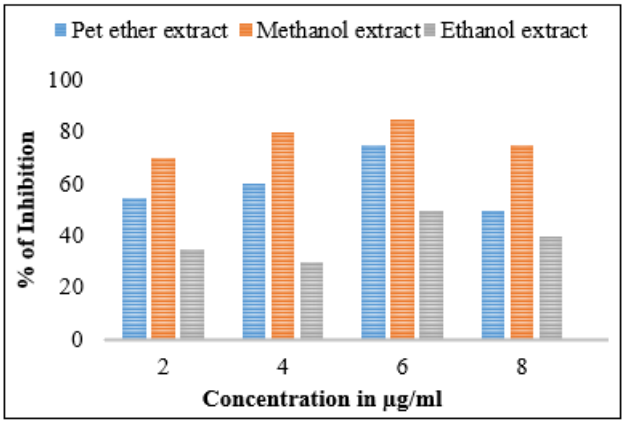



**Figure 5:** Graphical representation of % of inhibition observed in Nitric oxide Scavenging Activity

**Zone of inhibition for aqueous extracts**

Zone of inhibition formed from the aqueous extracts of *A. alata* Nees. at various concentrations. The zone of inhibition was found to be more against E. coli cultures. The similar type of aqueous extracts of some selected species are also carried out by Mudzengi *et al.*, 2017 [25].

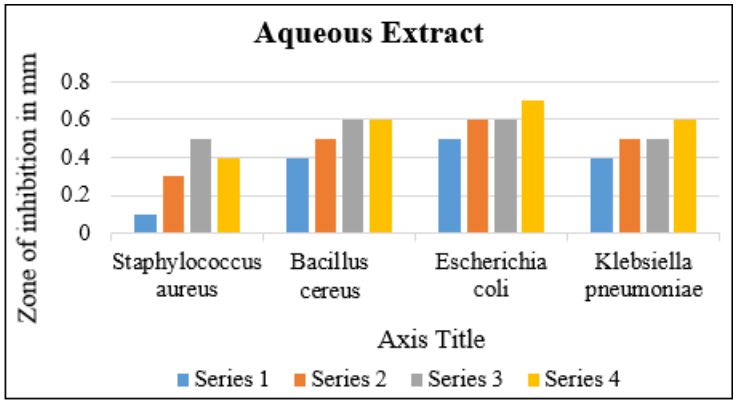



**Figure 4:** Indicating different concentration of aqueous extract for bacterial cultures

**Zone of inhibition for aqueous extracts**

Zone of inhibition formed from the aqueous extracts of *A. alata* Nees. at various concentrations

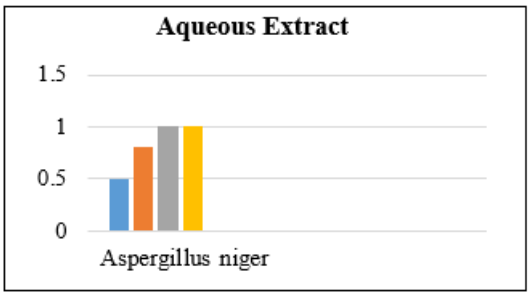



**Figure 4:** Indicating different concentration of aqueous extract for Fungal cultures

## Conclusion and Scope Future for Work

From the present investigation it is observed that, the plant contains potential antibacterial and antifungal components that may be of useful for evolution of pharmaceutical for the therapy of ailments. The petroleum ether, methanol and ethanol extracts of *A. alata* (Vahl) Nees. possess significant inhibitory effect against the tested organisms.

The current study is the first experimental demonstration of any biological properties as well as antibacterial and antifungal activity of *A. alata* (Vahl) Nees. Further studies are going on this plant in order to find the bioactive principles to develop new antibacterial and antifungal medications

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