International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Impact Factor (2012): 3.358

Phytochemical Analysis and Antimicrobial Evaluation of *Andrographis macrobotrys* Nees - An Endangered Medicinal Plant of India

C. Alagesaboopathi

Department of Botany, Government Arts College (Autonomous), Salem - 636 007, Tamil Nadu, India

Abstract: ***Andrographis macrobotrys*** **Nees is an extensively utilized Indian traditional medicine to treat several human and livestock disorders. The present investigation was designed to evaluate the antibacterial, antifungal potentialities and phytochemical screening of the different leaves and stem extracts of *Andrographis macrobotrys* belongs to the family Acanthaceae. Methanol, ethanol, chloroform, acetone and petroleum ether extracts of shade dried plant leaf and stem of *A. macrobotrys* were tested for antibacterial, antifungal properties and phytochemical analysis. The antibacterial potential of different extracts of leaves and stem of *A. macrobotrys* were assayed using the standard disc diffusion manner against five strains of bacterial species, viz., *Staphylococcus aureus, Pseudomonas aeruginosa, Proteus vulgaris, Klebsiella pneumoniae* and *Escherichia coli.* Among various solvent extracts evaluated, the petroleum ether leaf extract showed highest antibacterial assay against *Staphylococcus aureus* (18.11mm); followed by ethanol extract (15.41mm). The highest inhibition zone noted for methanol extract of *A. macrobotrys* leaves against *P.vulgaris* 15.13mm followed by chloroform extract (13.81 mm). The highest activity of acetone extract against *K. pneumoniae* was 12.90 mm. The chloroform leaf extract *A. macrobotrys* showed highest antifungal activity against *Alternaria alternata* (9.45 mm). The ethanol leaf extracts showed maximum activity against *Aspergillus niger* (9.10 mm). The methanol leaf extracts showed highest activity against *Aspergillus flavus* (8.15 mm). Acetone leaf extracts showed significant maximum activity against *Penicillium pinophilum* (7.39 mm). Petroleum ether leaf extracts showed maximum activity against *Fusarium solani* (7.11 mm). The leaf extracts showed great inhibitory effect than the stem extracts. Results showed the presence of saponins, flavonoids, triterpenoids, glycosides, gums and mucilages, steroids, tannins and phenolic compounds. This is the first report on antibacterial antifungal potentialities and phytochemical analysis of *A. macrobotroys.* This research scientifically validates the utilize of this plant as a potential antibacterial and antifungal properties.**

Keywords: *Andrographis macrobotrys*, Antifungal activity, Antibacterial activity, Phytochemical analysis, Medicinal Plant, Agar well diffusion method.

1. Introduction

India is endowed with a rich wealth of medicinal plants which have been a valuable source of natural products for maintaining human health. A large number of these medicinal plants are used in various formulations for the treatment of several diseases caused by microbes. According to World Health Organization (WHO), medicinal plants would be source obtaining a variety of drugs. Several societies across the world have shown great interest in curing ailments using plants / plant based drugs. Microbes are closely associated with the health and welfare of human beings. Some are beneficial and some are detrimental. As preventive and curative measures, plants and their products are used in the treatment of infections for several centuries ago. WHO estimated that 80 percentage of the people worldwide rely on plant based medicines for their primary health care needs [1,2] and India happens to be the largest user of traditional medical treat, using 7000 plant species.

The increasing failure of chemotherapies and antibiotic resistance exhibited by pathogenic microbial infections agent have led to the screening of various medicinal plants for their potential antimicrobial activity [3-5]. Antibacterial properties of various plants parts such as rhizomes, roots, stem, leaves, seeds and fruits have been well documented for some of the medicinal plants for the past two decades [6]. Antibiotic principles are distributed widely among angiospermic plants. A variety of compounds are accumulated in plant parts accounting for their constitutive antimicrobial properties. Herbal medicine represents one of the most important fields of traditional medicine all over the world. Many plants in the world are being tested for antimicrobial activities and the results derived from these scientific studies have aided in the validation of traditional uses of these plants [7]. The world is lush with naturally grown medicinal plants. In India, 500 medicinal plant species are used to pathogenic bacteria [8]. Plants have been used as traditional medicine since time immemorial to control bacterial, viral and fungal diseases.

Medicinal plants are now more focused than ever because they have the capability of producing many benefits to society indeed to mankind, especially in the line of medicine and pharmacological [9]. Plant based drugs have been in use against various diseases since time immemorial [10]. Any parts of plant: rhizome, root, stem, leaves, flowers, seeds and fruits etc. which have one or more of its organs constituents that can be utilized for therapeutic purposes, are called medicinal plants [11]. It is a fact that the 25% of all medical prescriptions are based on substances derived from plants or plant – derived synthetic analogues [12]. There is a great contribution of medicinal plants in the human healthiness for the treatment of different ailments. Plants are the main source of anti-infective agents like emetine, quinine and berberine that are highly effective instruments in the fight against microbial infections. Phytoremedies have also shown great promise in the treatment of intractable infectious

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diseases, including opportunistic AIDS infections [13]. The phytochemical constituents and medicinal properties of most of the medicinal plants were recorded in the last few decades by a number of workers [14-16]. These medicinal plants are subjected to several process and one then administrated to the patients. They survey and documentation of medicinally important plants in each and every place is very much important for easy identification of local traditional healers, conservation and sustainable utilization. Antibacterial and antifungal properties of medicinal plants parts such as leaf stem and root have been well noted for some of the medicinal plants for the past two decades. Medicinal plants and their essences are wealth antibacterial compounds can fight against bacterial ailments [17].

Indian medicinal plants and their products are used to control diverse disease such as catarrh, bronchitis, pneumonia, ulcers and diarrhoea. Researchers are increasingly turning their attention to folk medicine looking for new leads to develop better drugs against cancer, as well as viral and microbial infections [18,19]. Although hundreds of plant species have been tested for antimicrobial properties, the vast majority have not yet been adequately evaluated [20]. In every developing country it is necessary that the documentation of medicinal plants be treated as a matter of extreme urgency.

The search for plants with antimicrobial activity has gained increasing importance in modern years due to growing worldwide concern about the alarming increase in the rate of infection by antibiotic - resistant microorganism [21]. Medicinal plants would be the best source to obtain a variety of drugs [22]. As preventive and curative measures, plants and their products are used in the treatment of infections for many centuries before. WHO estimated that 80% people worldwide rely on plant based medicines for their main healthcare [23] and India happens to be the largest user of traditional medical treat, using 7000 plant species.

In addition to the alarming increase in the incidence of modern and re-emerging infectious diseases, one major health concern is the resistance to existing antibiotics [24]. Furthermore, novel antibiotics in the drug, development pipeline that offers significant benefits over existing drugs is lacking [25]. It is largely known that plants possess healing properties [26]. Such properties can be partly attributed to the diverse array of secondary metabolites (i.e., terpenes, alkaloids, phenolic compounds and cyanogenic glycosides) which are known to be essential for plants defense against microbial attack [27-29]. Treatment of common infections with medicinal plant has been familiar developing countries due to its cheaper cost and claims for both its effectiveness and lesser side effects over synthetic drug [30]. A diverse range of compounds that offer potentials for the treatment of chronic and infectious disease can be found most especially in traditional medicinal plants [31].

Well-known drugs that were derived from plants are taxol from *Taxus brevifolia* vinblatine and vincristine from *Catharanthus roseus*., benzoin from *Styrax tonkinensis* and quinine from *Cinchona pubscens* [32]. It is also well recognized that traditional medicine can be used along side synthetic pharmaceutical products for enchanged health management. Medicinal plants are an important source for the therapeutic remedies of various ailments. Scientific experiments on the antimicrobial properties of plant components were first documented in the late 19th century [33]. Natural antimicrobials have been often derived from plants, microorganisms or animal tissues [34]. India is known for its rich diversity of medicinal world [35]. Nearly 70 percent of the world population is dependant on the traditional medicines for primary health care. The knowledge of medicinal plants has been accumulated during the course of many centuries based on different medicinal systems such as Ayurvedha, Homeopathy, Naturopathy, Amchi, Modern, Unani and Siddha. In India it is reported that traditional healers used 2500 plant species and that 100 species of plants served as regular sources of medicine [36].

An antimicrobial is a substance that kills or inhibits the growth of microorganisms such as fungi, bacteria or protozoan's as well as destroying viruses [37]. Antimicrobial drugs either kill microbes or prevent the growth of plants with a new eye for their antimicrobial usefulness and as an alternative source to existing drugs. Plants with their wide variety of chemical constituents offer a promising source of new antimicrobial agents with general as well as specific activity [38]. There are many reports on the presence of antimicrobial compounds in various plants [39,40] but there are no reports on antimicrobial potential on *Andrographis macrobotrys*. Phytochemical from medicinal plants showing antimicrobial properties have the potential of filling this need, because their structure are different from those of the more studied microbial sources, and therefore their mode of action may too very likely differ [41]. There is growing interest in correlating the phytochemical constituents of a medicinal plant with its pharmacological activity [42]. Screening active compounds from plants has lead to the discovery of new medicinal drugs which have efficient protection and treatment roles against various ailments, including cancer [43].

Andrographis macrobotrys Nees is a member of the Acanthaceae family. Species of *Andrographis* Wallich ex Nees are used in the Indian systems of medicine such as Ayurveda, Homeopathy, Naturopathy, Siddha, Unani, Amchi and Modern [44]. The genus *Andrographis* as a whole is of potential importance to India. The genus exhibits antipyretic properties [45]. This genus consists of 40 species distributed in Tropical Asia [46]. About 21 species are distributed in India [47] and all of them available in Tamilnadu [48]. Among the 21 species 18 species are reported to be endemic to India [49]. *Andrographis macrobotrys* Nees is an endangered medicinal plant found in wild in Shevaroy Hills of Eastern Ghats, Salem district of Tamilnadu. ($11^{o}45'$ and $11^{o}55'$ latitude; $78^{o}11'$ to $78^{o}20'$ E longitude) upto 1400 m.

It is used to treat diarrhoea, muscular pain, fever, snake bite, antipyretic and skin diseases [50-52]. Two new flavonoids were isolated from the whole plant extract [53]. There is no previous report on antimicrobial activity of this plant could be found in literature.

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Plants generally produce several secondary metabolites which constitute an important source of microbicides, pesticides and many pharmaceutical drugs. Plant products still remain the principal source of pharmaceutical agents used in traditional medicine [54,55]. Phytoconstituents confer specific characteristics and properties to plants.

2. Materials and Methods

2.1 Plant Material Extraction

Leaf and Stem of *Andrographis macrobotrys* was collected from the Shevaroy Hills, Salem district of Tamilnadu. This plant was identified and confirmed with the authentic. A voucher specimen was deposited (No. CA/43/2013) in the Department of Botany, Government Arts College (Autonomous), Salem for the future reference. Fresh leaves and stem were washed thoroughly under running tap water and dried under shade. They were then finely ground to a powder in an electric blender. All parts were extracted with acetone, ethanol, methanol, petroleum ether and chloroform using soxhlet apparatus. After removal of solvents under reduced pressure, extracts were stored at -20°C until use. Then the extracts were used for antibacterial, antifungal and phytochemical analysis.

2.2 Antibacterial Assay

Antibacterial activity of all extracts from *Andrographis macrobotrys* were checked against *Escherichia coli, Staphylococcus aureus, Proteus vulgaris, Klebsiella pneumoniae* and *Pseudomonas aeruginosa.* The fungal strains used were *Penicillium pinophilum, Alternaria alternata, Aspergillus niger, Aspergillus flavus* and *Fusarium solani.* All the cultures were obtained in pure form from the Biomedical Engineering Research Foundation, Salem, Tamilnadu, India. Antibacterial assay was carried out by Agar well diffusion method [56,57]. Fresh microbial culture of 0.1ml having 10^8 CFU was spread on nutrient agar plate with glass spreader. A well of 6 mm diameter was punched off into agar medium with sterile cork borer and filled with 50 µg of ethanol, methanol, acetone, chloroform and petroleum ether extracts by using micropipette in each well in aseptic condition. The petriplates were then kept in a refrigerator to allow pre-diffusion of extract for 30 minutes and further incubated in a incubator at 37°C for 24 h. The antibacterial screening was evaluated by measuring the zone of inhibition. The experiment was done in triplicate and the mean diameter of the inhibition zone was calculated. Antibiotic ciprofloxacin at a concentration of 25 µg/ml as positive control and 100% dimethylsulfoxide (DMSO) as a negative control were used.

2.3 Phytochemical Analysis

The ethanol, acetone, methanol, petroleum ether and chloroform extracts of *Andrographis macrobotrys* were screened for the presence of secondary metabolites using the procedures of Harborne [58] and Kokate *et al.*, [59]. The leaf and stem extracts was assayed for the presence of glycosides, flavonoids, gums and mucilages, steroids, triterpenoids, tannins, saponins and phenolic compounds.

2.4 Antifungal activity

The method of Bauer *et al.*, 1996, was adopted for the study. Antifungal activities of leaf and stem of *Andrographis macrobotrys* was proved in a radical growth inhibition activity. A fungal plug was placed in the center of the Potato Dextrose Agar plate. Extracts of 25 mg/ml concentrate was pipetted into the wells. The petriplates were incubated in the dark at 23°C for 48 h. Antifungal properties was observed as a cresent shaped zone of inhibition at the mycelial form. The effect of fungal growth was expressed qualitatively. Comparison of antifungal activity of various extracts was done with standard antifungal fluconazole at a concentration of 25 µg as a positive control. The diameters of zone of inhibition surrounding each of the well were recorded.

2.5 Statistical analysis

Agar well diffusion activity was performed in three replicates under strict aseptic conditions to ensure consistency of all conclusions. Data of all experiments were statistically analysed and expressed as Mean ± Standard Deviation.

3. Results and Discussion

The determinations from the present investigation showed that the five leaf and stem extracts (methanol, ethanol, acetone, petroleum ether and chloroform) of *Andrographis macrobotrys*, revealed antibacterial properties against all the five human pathogens tested. As noticed from Table 1, All the extracts exhibited broad spectrum of activity. When the five extracts were compared with each other and with that of standard antibiotic ciprofloxacin, the chloroform leaf extract observed to have highest potential compared to that of the acetone, methanol, ethanol and petroleum ether extracts.
The investigation made on chloroform extract highest activity against *Escherichia coli* (17.05 mm) *Proteus vulgaris* (13.81mm) and *Staphylococcus aureus* (13.24 mm) and least inhibition zone was observed against *Klebsiella pneumonia* (8.30 mm) and *Psudomonas aeruginosa* (10.43 mm). Where as no activity pathogen like *Staphylococcus aureus* in chloroform stem extract. The extracts using petroleum ether showed highest inhibition zone observed against *Staphylococcus aureus* (18.11 mm) and *Klebsiella pneumonia* (11.21 mm) and the minimal activity against *Escherichina coli* (8.20 mm). It has no activity against pathogen like *Pseudomonas aeruginosa, Proteus vulgaris* (stem extract) and *Klebsiella pneumoniae* (leaf extract). Acetone extract pointed out maximum activity against *Staphylococcus aureus* (13.10 mm) and *Klebsiella pneumoniae* (10.32 mm). Showed that least activity against *Escherichia coli* (8.13 mm) and *Proteus vulgaris* (8.15 mm). The extract obtained using methanol showed a highest activity against pathogen like *Pseudomonas aeruginosa* (11.0 mm) and *Escherichia coli* (10.61 mm). Observed no activity against pathogen like *Staphylococcus aureus* (leaf extract. The ethanol extract antimicrobial activity results showed diameter of inhibition zones ranging from (8.40 to 15.41 mm), with the highest zone of inhibition shown

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towards *Staphylococcus aureus* (15.41 mm). Least inhibition zone was observed against *Klebsiella pneumoniae* (8.40 mm). Where it has no activity against *Escherichia coli* in stem extract (Plate-1 and Plate-2)

There is no previous report on evaluation of this plant concerning its antibacterial activity. Although, the antibacterial effect of another species of this family (Acanthaceae) has been reported [60,61]. Table No.2 displayed the antifungal activity of leaves and stem extracts of *A. macrobotrys*. The results of minimum inhibitory concentrations (MIC) study proved the antifungal activity of extracts against the tested strains of microorganisms. Antifungal activity denoted that the tested fungal strains are most susceptible to chloroform extract. Chloroform extract antifungal results showed the diameter of inhibition zones ranging from 6.0 to 9.45 mm with the highest inhibition zone observed against *Alternaria alternata* (9.45 mm). Minimal inhibition zone was noticed against *Aspergillus flavus* (6.0 mm). It has no activity against *Penicillium pinophium*. Observation made from methanol extract showed a highest activity against *Aspergillus flavus* (8.15 mm), *Alternaria alternata* (7.51 mm) and *Fusarium solani* (7.41 mm) and the minimum activity against *Aspergillus niger* (6.44mm). Where as no activity against *Penicillium pinophilum*. Petroleum ether extract antifungal results observed the diameter of inhibition zone noticed against *Fusarium solani* (7.11 mm). Least inhibition zone was showed against *Penicillium pinophilum* (5.46 mm. Showed no activity against *Alternaria alternata*. The ethanol extract observed highest activity against *Alternaria alternata* (9.20 mm) and the minimum activity against *Penicillium pinophilum* (7.0 mm). Where it has no action against *Fusarium solani*. The extract obtained using acetone showed a highest activity against *Penicillium pinophilum* (7.39 mm) minimal inhibition zone was observed against *Alternaria alternata* (5.70 mm). Where it has no activity against *Fusarium solani* and *Aspergillus flavus*.

The present research was to investigate the leaf and stem samples revealed the presence of medicinally bioactive constituents. The phytochemical analysis of *A.macrobotrys*investigated are presented in Tables 3 and 4. Qualitative phytochemical test for methanol, ethanol, chloroform, acetone and petroleum ether extracts of the drug carried out. The phytochemical analysis of the different extracts from the leaf and stem sample of *A. macrobotrys* revealed the presence of phytochemicals such as saponins, flavonoids, triterpenoids, glycosides, gums and mucilages, tannins, phenolic compounds and steroids. The presence of these phytoconstituents suggests that the plant might be of medicinal importance and pharmaceutical industrial. The phytoconstituents like carbohydrates, alkaloids, protein and amino acids were absence in leaf and stem sample of *A. macrobotrys* (Table 3).

Table 4 shows quantitative estimation of the percentage phytochemicals of *A.macrobotrys. A. macrobotrys* contained the highest percentage yield of flavonoids (18.43%) in chloroform leaf extract. The content of gums and mucilages was found highest (14.17%) in chloroform leaf extract. *A. macrobotrys* contained the lowest yield of phenolic compounds (0.09%) but the highest percentage yield of tannin (7.10%). Triterpenoids were obtained in the plant but the yields recorded were minimal (0.30 - 0.10%). Saponin high yield of 3.45% and lowest yield was found in 1.55%. Steroids were obtained in the plant but the yields recorded (0.51 - 0.29%). The content of glycosides was found in *A. macrobotrys* (0.45 - 0.20%). The phytochemical research and quantitative estimation of the percentage yields of chemical constituents of the plant studied that the leaves and stem were rich in flavonoids, gums and mucilages saponins and tannins. The were known to show medicinal activity as well as exhibiting physiological properties [62]. Saponin has the activity of precipitating and colligating red blood cells. Saponin also foams in aqueous solutions, hemolytic properties and bitterness, flavonoid on the other hand, are effective water soluble anti-oxidants activity with prevent oxidative cell damage, have potent anti-cancer properties. The phytochemicals are known to have antimicrobial screening [63]. The phytochemical screening revealed the presence of the saponins, flavonoids, tannins, steroids, triterpenoids, phenolic compounds, gums and mucilages respectively. It can be suggested that *A. macrobotrys* are not only interesting source of medicinal properties but also potential source of phytoconstituents.

It is concluded the present study the plant contains potential antibacterial and antifungal components that may be of beneficial for evolution of pharmaceutical for the therapy of ailments. The acetone, chloroform, petroleum ether, methanol and ethanol extracts of *A. macrobotrys* leaf and stem possess significant inhibitory effect against the tested organisms. The results of the investigation support the traditional claimed of this plant. Apart from this investigation, there are no reports of antibacterial, antifungal and phytoconstitutents studies of *A. macrobotrys*. The current study is the first experimental demonstration of any biological properties as well as antibacterial and antifungal activity of *A. macrobotrys*. These results also assist helpful health maintains. Thus, there is huge scope for future investigation and future pharmacological research on *A.macrobotrys.*

4. Acknowledgments

The Author C. Alagesaboopathi is very greatful and thankful to Tamilnadu State Council for Higher Education, Chennai, Tamilnadu, India [TANSCHE Letter No.D.O.Rc.No.570/2012A] for providing financial assistance of this Minor Research Project Scheme for Teachers Research Project work.

Table 1: Antimicrobial activity of leaves and stem extracts of *Andrographis macrobotrys* Nees

Plant part	Plant Extracts	Zone of inhibition (mm)				
		Escherichia coli	*Proteus vulgaris*	*Staphylococcus aurues*	*Pseudomonas aeruginosa*	*Klebsiella pneumoniae*
Leaves	Acetone	10.24 ± 0.07	8.15 ± 0.18	13.10 ± 0.22	9.0 ± 0.12	12.90 ± 0.50
	Methanol	9.11 ±	15.13 ±	-	11.0 ±	10.33 ±

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		0.16	0.50		0.15	0.44
	Ethanol	11.62 ± 0.14	12.13 ± 0.44	15.41 ± 0.18	10.03 ± 0.18	12.65 ± 0.63
	Petroleum ether	10.08 ± 0.06	11.0 ± 0.15	18.11 ± 0.05	-	-
	Chloroform	17.05 ± 0.04	13.81 ± 0.07	13.24 ± 0.33	10.05 ± 0.70	10.51 ± 0.28
Stem	Acetone	8.13 ± 0.07	9.12 ± 0.20	10.30 ± 0.41	8.15 ± 0.10	10.32 ± 0.04
	Methanol	10.61 ± 0.04	8.41 ± 0.11	9.11 ± 0.03	8.60 ± 0.22	9.37 ± 0.05
	Ethanol	-	10.33 ± 0.60	11.30 ± 0.09	10.19 ± 0.81	8.40 ± 0.02
	Petroleum ether	8.20 ± 0.08	-	10.03 ± 0.08	-	11.21 ± 0.15
	Chloroform	9.42 ± 0.15	10.50 ± 0.70	-	8.43 ± 0.17	8.30 ± 0.21
	Ciprofloxacin (25 µg/m1)	24.0 ± 0.12	26.0 ± 0.08	23.0 ± 0.19	26.0 ± 0.05	27.0 ± 0.40

Data given are mean of three replicates ± Standard error.
:- No inhibition
Concentration used 50µg/m1

Table 2: Antimicrobial activity of leaves and stem extracts of *Andrographis macrobotrys* Nees

Test organisms	Methanol extract		Ethanol extract		Chloroform extract		Acetone extract		Petroleum ether extract		Fluconazole (25µg)
	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	
Alternaria alternata	7.51± 0.13	6.20± 0.50	6.20± 0.40	0 ± 00	9.45± 0.11	6.10± 0.71	6.61± 0.37	5.70± 0.03	5.33± 0.49	0.00	15±0.10
Fusarium solan	7.41± 0.22	7.04± 0.14	0.00	6.41± 0.31	8.11± 0.40	7.33± 0.10	0.00	6.70± 0.34	7.11± 0.66	6.10± 0.28	14±0.17
Aspergillus flavi	8.15± 0.15	6.20± 0.40	7.90± 0.15	7.0± 0.10	8.0± 0.30	7.10± 0.05	6.11± 0.31	0.00	6.9± 0.70	6.0± 0.68	17±0.20
Aspergillus niger	6.44± 0.30	6.11± 0.11	9.10± 0.20	7.20± 0.41	8.70± 0.25	6.0± 0.44	7.14± 0.63	6.90± 0.38	7.0± 0.50	6.33± 0.19	15±0.36
Penicillium pinophilum	0.00	6.80± 0.57	7.0± 0.35	0±00	7.10± 0.60	0.00	7.39± 0.71	6.13± 0.25	6.41± 0.05	5.46± 0.07	16±0.05

Table 3. Phytochemical analysis of the leaf and stem of *Andrographis macrobotrys* Nees.

Phyto-constitutents	Methanol extract		Ethanol extract		Chloro-form extract		Acetone extract		Petroleum ether extract	
	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem
Saponins	+	+	+	+	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+	+	+	+	+
Triterpenoids	+	+	+	+	+	+	+	+	+	+
Alkaloids	–	–	–	–	–	–	–	–	–	–
Glycosides	–	+	+	–	+	–	+	–	–	+
Gums & Mucilages	+	+	+	+	+	+	+	+	+	+
Steroids	–	–	+	+	–	–	–	+	–	–
Carbohydrates	–	–	–	–	–	–	–	–	–	–
Protein & Amino acids	–	–	–	–	–	–	–	–	–	–
Tannins	+	+	+	+	+	+	+	+	+	+
Phenolic compounds	+	+	+	+	+	+	+	+	+	+

Key: (+) - Present (–) - Absent

Table 4: Phytochemical composition of *Andrographis macrobotrys* Nees.

Phyto-constitutent	Methanol extract		Ethanol extract		Chloroform extract		Acetone extract		Petroleum ether extract	
	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem
Saponins (%)	3.41± 0.12	1.70± 0.20	3.51± 0.33	1.90± 0.07	2.90± 0.27	1.55± 0.46	3.10± 0.15	1.82± 0.6	3.45± 0.19	1.86± 0.20
Flavonoids (%)	14.30± 0.14	7.10± 0.30	12.70± 0.04	6.40± 0.13	18.43± 0.60	8.11± 0.18	13.60± 0.37	7.20± 0.07	15.40± 0.88	7.08± 0.12
Triterpenoids (%)	0.24± 0.6	0.10± 0.07	0.19± 0.18	0.15± 0.22	0.30± 0.36	0.12± 0.22	0.22± 0.40	0.14± 0.31	0.26± 0.16	0.13± 0.50
Alkaloids (%)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Glycosides (%)	0.00	0.36± 0.18	0.45± 0.30	0.00	0.24± 0.11	0.00	0.20± 0.26	0.00	0.00	0.21± 0.30
Gums and Mucilages (%)	13.40± 0.19	6.90± 0.11	10.81± 0.35	8.20± 0.10	14.17± 0.66	8.50± 0.22	12.71± 0.47	6.80± 0.09	11.65± 0.15	8.16± 0.30
Steroids (%)	0.00	0.00	0.51± 0.24	0.29± 0.08	0.00	0.00		0.34± 0.19	0.00	0.00
Carbohy-drates (%)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

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Protein and Amino acids (%	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Tannins (%)	6.20± 0.27	2.13± 0.16	5.40± 0.28	1.75± 0.40	7.10± 0.55	2.40± 0.1	5.11± 0.65	2.06± 0.33	6.15± 0.48	1.95± 0.36
Phenolic compounds (%	0.88± 0.17	0.09± 0.30	0.45± 0.25	0.22± 0.11	0.65± 0.42	0.28± 0.15	0.41± 0.34	0.20± 0.10	0.91± 0.23	0.39± 0.65

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Author Profile



Dr. C. Alagesaboopathi has authored one book Endemic Medicinal Plants. He has published 112 research papers in National and International reputed Journals. He has guided 60 students for their M.Phil, Degree in the field of Botany, Microbiology and Biotechnology and 1 Ph.D. scholar in the field of Biotechnology. Now he has working as Assistant Professor, Department of Botany, Government Arts College, Salem, Tamilnadu, India.

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