Scientific Foundation SPIROSKI, Skopje, Republic of North Macedonia
Open Access Macedonian Journal of Medical Sciences. 2025 Mar 15; 13(1):61-76.
https://doi.org/10.3889/oamjms.2025.12018
eISSN: 1857-9655
Category: *A – Basic Sciences*
Section: *Pharmacology*

Harnessing the Phytochemical, Pharmacological and Anti-Microbial Potentials of Andrographis Alata (Vahl) Nees

Ruth Sylvia AC[1], Sumathi P[1], Abdulrahman Jadid Alanazi[2], Ibrahim Tarik Munir Aljundi[2], Turki Azi Hazazi[2], Mohamed Ali-Seyed[2*]

[1]Postgratuate and Research Department of Botany, Kongunadu Arts and Science College, Coimbatore, 641029, Tamil Nadu, India; [2]Department of Biochemistry, Faculty of Science, University of Tabuk, Tabuk, 71491, Kingdom of Saudi Arabia

Edited by: Ksenija Bogoeva-Kostovska
Citation: Sylvia ACR, Sumathi P, Alanazi AJ, Aljundi ITM, Hazazi TA, Ali-Seyed M. Harnessing the Phytochemical, Pharmacological and Anti-Microbial Potentials of Andrographis Alata (Vahl) Nees. Open Access Maced J Med Sci. 2025 Mar 15; 13(1):61-76. https://doi.org/10.3889/oamjms.2025.12018
Keywords: A. alata; Phytochemicals; Natural Products; Phytochemical Screening; Antioxidant; Antimicrobial
***Correspondence:** Mohamed Ali-Seyed. Department of Biochemistry, Faculty of Science, University of Tabuk, Tabuk, 71491, Kingdom of Saudi Arabia.
E-mail: Mohamed Ali-Seyed
Received: 02-Feb-2025
Revised: 14-Feb-2025
Accepted: 05-Mar-2025

Funding: This research did not receive any financial support
Competing Interests: The authors have declared that no competing interests exist

Abstract

BACKGROUND: Nature has been a soul source of therapeutic pharmaceutics for a long time and a notable number of advanced pharmaceutics have been invented from this source. Numerous drug leads were identified with the contribution of combinatorial and other allied field experts in the traditional medicine field. The World Health Organization (WHO) characterizes complementary medication as the “wide range of heath practice approaches for prevention, diagnosis and treatment”. However, there is an urgent necessity to validate the obtained information by employing various advanced techniques and tools before it has applied for drug discovery or as effective therapeutics in the clinic along with existing therapies.

AIM: Hence, vigorous investigations on herbal plants are required to assess their pharmacological properties and efficacy. India is one of the richest emporia of herbal plants and termed as ‘Botanical Garden’ of the world because of its long history in the traditional knowledge on herbal plants and significant contribution and application in the modern medical practice. Ethno pharmacological evidence measured as an effective tool in the novel discovery of novel drug leads from herbal plants and is a pre-requisite for the practice of plant derived active secondary metabolites. The members of the Acanthaceae family have therapeutic importance owing to the existence of many active secondary bioactive principles or novel phytoconstituents.

MATERIALS AND METHODS: The present study embarked into Andrographis alata (AA), one of the less explored but well prescribed traditional plants and validated its medicinal properties using various techniques.

RESULTS: Since, other species of Andrographis, possessed ample alkaloids and diterpenoids which exhibited varied therapeutic properties which include pharmacological and antimicrobial activities, this AA less investigated multiple aspects including its phyto-screening.

CONCLUSION: The phytochemicals obtained from AA with its multifaceted activities may serve the ideal therapeutic as well as an analogue for many combinatorial drugs. This study also justified the traditional values in the complementary system and therapeutic values. However, further investigations on its various bioactive principles and their mechanism of action are highly warranted.

Introduction

Medicinal plants are recognized for their curative potential for millennia and are highly admired globally as a reservoir of therapeutic pharmaceuticals for the management of human illnesses [1], [2], [3], [4]. Herbal medicine is defined as medicinal herb-based preparations are in place to cure diseases [4], [5], [6].

In recent years, herbal therapy coined as a more precise synonym of complementary medicine [7], [8]. Despite the advantage of synthetic medicines and antibiotics, the importance and demand for herbal derivatives were reduced in the developed nations, however, recent years witness there is a changing in paradigm shift from synthetic to herbal medicine and termed now as “Return back to Nature’s gift as drugs [9], [10], [11].

In recent years, the importance of medicinal herbs place in traditional medical practice are gaining increasing attention [4], [5], [6], [9]. The resurgence of interest in herbal plants is growing phenomenally worldwide more specifically in the developing countries as they have accepted traditional medicine as an integral part of their culture [12]. This recent renaissance of attention in plant-based remedies spurred on by several factors [13], [14], [15]. Drug discovery from medicinal plants typically involves with

multiple expertise by employing screening assays 16 in which phytochemists separate extracts from the plant materials, which are subjected to various stringent biological and pharmacological screening using an appropriate advanced assays followed by isolation of active drug leads through bioassay-guided fractionation [17], [18].

The tremendous growth of therapeutic drug industry has an incredible influence on the management of various human diseases and prevention [19]. Although, complimentary medicine had great impact on infectious diseases control, yet still some concerning factors exists like many microbial organisms exhibit more resistance to antibiotic treatments [20] and the serious side effects brought by many combinatorial synthetic drugs. The aforesaid reasons gained renewed attention towards herbal plants as a source of novel pharmaceuticals [21] Hence, an urgent necessity to authenticate the herbal plants wealth of data through an organized set-up to apply an effective pharmaceutics along with the current treatments or use as an instrument for drug discovery.

Andrographis belonging to Acanthaceae family and considered as one of the vital medicinal herbs. There are roughly 4000 species and 250 genera in the Acanthaceae family is referred to as the "king of bitters". The Andrographis family is comprised of [29] different species, which include A. alata, A. paniculata, A. longipedunculata, A. elongata, A. gracilis, A. affinis, and A. macrobotrys [9], [22]. There are many known medicinal attributions proposed for this family plants [23], [24] as they contain many bioactive principles such as saponins, flavonoids, quinones, phenols, terpenoids, tannins, and heart glycosides [25].

AA (Vahl) Nees is an herbaceous therapeutic plant species, distributed in various regions of South India [26]. This plant species is known to contain a diverse range of flavonoid glycosides, which include 5,7,2',6'-oxygenated flavone glycosides and 5,2',6'-trihydroxy-7-methylflavone-2'-O-ß-D-glucopyronoside [27], [28], [29], [30]. Previous report revealed that AA contains approximately 33.21 mg/g DW of neoandrographolide, which has been proven to possess various potent effects against oxidants, inflammation, malaria, and involved in liver protection [31], [32], [33]. One of the reasons for AA's less exploration on its medicinal properties maybe due to the decline its presence in natural populations can be attributed to the destruction of its habitat and excessive exploitation [34], [35], [36].

Andrographis species have various roles in traditional medicine [11], [34]. They are commonly used for snake bites, skin illnesses, and also for the treatment of jaundice, diabetes and employed as veterinary medicine [37], [38] and utilized as a laxative [39], an antibiotic [40], and a hepatoprotective [41]. The leaves of these species contain huge amounts of andrographolide, an alkaloid known to alleviate pain from colic, loss of appetite, and vomiting [42] and enhance immunity to treat various viral diseases [43]. Besides, AA offers numerous benefits against angiogenesis, cancer, inflammation, oxidants and exhibit immunostimulant, hepatoprotective, and insecticidal effects [44], [45], [46].

Excessive surge of radical oxidants, which comprise both reactive oxygen species (ROS) and reactive nitrogen species (RNS) result into oxidative burst or stress [47]. This stress causes changes in biomolecules, resulting in structural and functional modifications within the body. It is a pricipal factor in the development of numerous illnesses like liver disease [48], asthma [49], tumor [50] long ailing chronic inflammatory conditions like multiple sclerosis (MS) [51], [52] neural syndromes [53] rheumatoid arthritis (RA) [51], [52] cardiovascular disease (CVD) [53] aging [54] etc.

On the other hand, he plant kingdom serves as a reservoir of natural antioxidants that are essential in the food chain by combating free radicals and preventing the formation of singlet-oxygen [55]. Natural products containing antioxidants are utilized to support the endogenous system, with a growing interest in their oxidant quenching and nutraceutical roles [56]. In today's context, industry made combinatorial artificially synthesized antioxidants like Butylated Hydroxytoluene (BHT) and Butylated Hydroxyanisole (BHA) are pivotal in food processing industry, effectively mimicking the role of natural antioxidants [57], [58], [59].

Furthermore, therapeutic herbs are also a resource for several anti-microbial agents36, 60 as they dis-play bacteriostatic and fungicidal properties [61], [62] A diverse range of plant families like Asteraceae, Eu-phorbiaceae, Apocynaceae, Fabaceae, Leguminoceae and Rutaceae have yielded numerous products exhibiting antibacterial properties [63]. Despite the vast available data obtained from these plants on anti-microbial properties [64] till date no breakthrough occurred, or key antimicrobial drug lead have evolved from higher plants. However, wealthy of current and past results indicate that medicinal plant derived drug leads, even if they are not fully developed as mainstream antimicrobial drugs, they deserve to be incorporated into primary health care systems as an alternatives [65] because higher plants are not fully explored and their ethno-pharmacological information are not properly utilized to develop new drug leads [66], [67] but the data on herbal plants have proven of their substantial importance leading to the discovery of a new drug leads howev-er, they are yet to be explored in detail. In view of the above, it is imperative to investigate one of the very im-portant members of Acanthaceae family, AA, a potent herbal plant, which is either less investigated or unex-plored so far, hence, we need to evaluate its rightful potential biological activities and its multifaceted efficacy including pharmacological and anti-microbial activities.

Plant description

Systematic taxonomy description of Andrographis alata (Vahl) Nees Plant (Bentham and Hooker's system of classification, 1862-1883) [68].

Kingdom:	Plantae
Subkingdom:	Tracheophytes
Order:	Lamiales
Family:	Acanthaceae
Genus:	Andrographis
Species:	alata
Plant Name:	Andrographis alata (Vahl) Nees
Synonyms:	Ionoxalis attenuate Small.
Vernacular Name:	In Tamil: Perianangai

Medicinal attribution of Acanthaceae

The medicinal properties of Acanthaceae herbs are attributed to their richness in secondary bioactive principles such as flavonoids, quinones, cardiac glycosides, saponins, alkaloids, phenols, terpenoids and proteins. These compounds are employed as therapeutics to cure various diseases which include inflammation, kidney disorders, pyrexia, diabetes, heart ailments, and different types of cancers [5], [69], [70]. In addition, some species within the Acanthaceae family exhibit antibacterial properties, such as Berleria priorities, Adathoda zeylanica Neelagirianthasis hemitomie, Adathoda beddomie, Justeceae gendurusa, and Hemigraphis colorata shown effective actions against Pseudomonas species [71], [72]. Andrographis paniculata has shown promise as a potential treatment for severe acute respiratory syndrome (SARS-CoV-2; Covid-19).33, 73 Moreover, tra-ditional uses of plants from this species like Andrographis paniculata, Barleria prionitis, Hygrophila spinosa, and Adathoda vasica have demonstrated their action against pyretic, asthma, viral, and respiratory diseases [12].

Habitat of Andrographis alata

Andrographis alata found mostly in the tropical Asian nations like India and Srilanka [43]. In India, the plant widely distributed in South states like Tamil Nadu in Salem District, in Kerala Palakkad District, in Karnataka state Hassan and Kodagu (Coorg) and Mysuru District and in Andhra Pradesh Chittoor and Vishakapatnam Districts. The plant species AA subjected in this study identified and authenticated by the Botanical Survey of India, Southern Regional Centre, Coimbatore, 641003.

Materials and Methods

Plant material extraction

Fresh leaves of AA thoroughly washed in the running water to eliminate dust and other contaminations followed by dried at RT for a week. Then the samples pulverized into fine powder and stored for further experiments. The fine powder of leaf and stem materials from AA placed individually in a tiny container, which is subjected to isolation of extracts using various solvents like Ethyl Acetate, Ethanol and Petroleum ether in or-der of increasing polarity using a Soxhlet apparatus [17], [74], [75].

Secondary bioactive principles

Phytochemicals refer to bioactive principles found in several plant parts like grains, vegetables, fruits and other plant-based foods that are associated with reducing or inhibiting risk of major chronic diseases. These bioactive metabolites are categorized into phenolic, alkaloids, carotenoids, nitrogen-based and organo-sulfur compounds [76], [77]. Among these, phenolics and carotenoids are the most extensively studied [78]. Phenolic compounds contain more than one aromatic-ring with additional hydroxyl groups, and are typically classified into coumarins, tannins, flavonoids, stilbenes and phenolic acids [76], [77]. These bioactive metabolites produced as part of the secondary metabolism in plants and play a crucial role in reproductive process, defense mechanisms against various microbes, predators and parasites and have a role in plant pigmentation. In addition, phenolic compounds may also offer health benefits by reducing the risk of chronic diseases [79].

Besides, flavonoids, a subgroup of phenolic compounds, exhibit antioxidant properties. There are over 4,000 distinct flavonoids identified to date. These compounds typically share a basic structure comprising 2 aromatic rings (A and B rings) connected by three carbons forming heterocycle ring, or oxygenated C ring. Variations in the structure of heterocycle C ring differentiate them into flavanols (kaempferol, quercetin, and myricetin), flavones (like luteolin and apigenin), flavanols (including catechin, epicatechin, epigallocatechin gallate, and epigallocatechin gallate), flavanones (such as naringenin), anthocyanidins, and iso-flavonoids (like genistein) which are commonly found in the human diet [80]. In fact, A. paniculata, a promi-nent member of androgrphis produces substantial amounts of phytocompounds like diterpenoids, flavonoids, quinic acids, xanthones, and noriridoids [43]. The key diterpenoids found in this plant, including andro-grapholide, deoxyandrographolide, neoandrographolide, 14-deoxy-11, 12-didehydroandrographolide, and iso-andrographolide, all of them reveal different beneficial effects like liver and neuroprotective, immunostimu-lant. Additionally, these compounds exert against angiogenesis, atherosclerosis, tumor, diabetes, in-flammation, free radical oxidants and exhibit insecticidal properties [44], [45], [81].

Detection of bioactive principles of A. alata

The bioactive components of AA identified through the utilization of various standard methods82

to analyze the leaf and stem powder (Table 1). The major phytochemicals detected included carbohydrates, amino acids, proteins, alkaloids, flavonoids, phenolic compounds, tannins, glycosides, saponins, steroids, terpenoids, and cardiac glycosides [74], [82], [83], [84], [85], [86].

Table 1: Detection of major phytochemicals of *A. alata* leaf and stem extracts by employing various methods

S.No	Detection of major phytochemicals of A. alata	Method adopted	References
1	Detection of Carbohydrates	Molish's test	Ramakrishnan et al., 1994)
2	Detection of Proteins	Biuret test	Gahan, 1984)
3	Detection of Amino acids	Ninhydrin test	Yasuma and Ichikawa, 1953)
4	Detection of Alkaloids	Hager's test	Wagner et al., 1996)
5	Detection of Flavonoids	Alkaline reagent test	Raaman, 2006)
6	Detection of Phenolic	Ferric chloride test	Mace, 1963)
7	Detection of Tannins	Potassium hydroxide test & Gelatin's test.	Odebiyi and Sofowora, 1978; Williamson et al., 1996)
8	Detection of Glycosides	Borntrager's test	Evans, 1997)
9	Detection of Saponins	Frothing test	Kokate, 1999)
10	Detection of Terpenes	Libermann Burchard's test.	Sofowara, 1993
11	Detection of Cardiac glycosides	Keller Killiani test	Ngbede et al., 2008)
Quantification of Secondary Metabolites of A. alata			
12	Quantification of total phenolics	Siddhuraju and Becker (2003).	
13	Quantification of total Tannins	Siddhuraju and Manian, 2007	
14	Quantification of total flavonoids	Zhishen et al. (1999).	

In vitro antioxidant assays

The DPPH radical quenching property assessed to determine the antioxidant activity of selected sample extracts by measuring their capability to contribute hydrogen or scavenge radicals using the stable radical DPPH, according to the method described by [88], [89]. Besides, superoxide radical scavenging activity meas-ured by evaluating the ability of various extracts which prevent formazan formation by scavenging the super-oxide radicals generated in the riboflavin–light–NBT system, as described by [90]. The scavenging activity of superoxide calculated by this formula (below):

$$\text{Scavenging ability (\%)} = [(\text{Control OD} - \text{Sample OD})/\text{Control OD}] \times 100$$

The antioxidant activity of the AA samples was determined using the phosphomolybdenum assay, which involves the formation of phosphomolybdenum green complex [91]. This study used ascorbic acid as a reference standard. The results expressed as milligrams of ascorbic acid equivalents (AAE)/g extract.

The anti-bacterial potential of AA leaf and stem extracts investigated against various pathogenic bacte-rial strains donated by Bharathiar University, Coimbatore, Tamil Nadu, India. The following bacterial strains employed for this such as Acinetobacter baumannii, Bacillus cereus, Bacillus subtilis, Staphylococcus epidermis, Staphylococcus pyogenes, Enterobacter cloacae, Escherichia coli, Klepsiella pneumonia, Pseudomonas aeruginosa, and Staphylococcus aureus. The effect of various leaf and stem extracts on these bacterial strains evaluated using the Agar well disc diffusion method, as outlined by [92], [93]. For anti-fungal activity, the well diffusion test followed [94].

Statistical Analysis

Statistics analysis of data are presented as the results of 4 independent experiments. All statistical analysis performed using SPSS (IBM SPSS Statistics 25). One-way ANOVA analysis and student's t-test used to assess the study data. The results presented as means ± SD to illustrate differences across the experiments. Sta-tistical significance was determined at $P < 0.05$.

Results

The extract yield percentage of AA leaves and stems presented in Table 2. In fact, the maximum yield observed in stems with ethanol extract (15.08%) compared to leaf extract (14.2%). The second highest yield is shown by ethyl acetate extract in the stem (8.4%).

However, the yield percentage of petroleum ether and aqueous extract was low when compared to other solvents. Our results propose that the high polar solvents dissolve more constituents found in leaf and stem parts of AA.

Table 2: Extracts yield percentage of *A. alata* leaf and stem

S. No.	Solvents	Extract yield percentage	
		Leaf	Stem
1	Petroleum ether	2.9	2.62
2	Ethyl acetate	5.44	8.4
3	Ethanol	14.2	15.08
4	Aqueous	2.3	3.67

The qualitative detection/estimation of phytochemicals was carried out in various extracts of leaf and stem of AA to identify the presence of principal and secondary bioactive principles (Table 3).

The results revealed that the presence of primary metabolites like carbohydrates, proteins and amino acids in all the extracts of leaves and stems. The secondary bioactive principles like alkaloids, flavonoids, phenol, saponins and cardiacglycosides are variously distributed in the selected leaf and stem extracts.

However, tannin, terpenoids and glycosides were found to be absent in petroleum ether (PE) and ethyl acetate (EA) extract of both the leaf and stem samples. In addition, steroids were found to be absent from all the extracts.

Table 3: Qualitative phytochemical analysis of A. alata leaf and stem extracts. (+): Presence of chemical compound, (-): Absence of chemical compound (+) < (++) < (+++): Based on the intensity of characteristic colour

Phytochemicals	Petroleum ether extract		Ethyl acetate extract		Ethanol extract		Aqueous extract	
	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem
Carbohydrate	++	++	++	+++	++	++	++	++
Protein	++	++	++	++	++	++	++	++
Amino Acid	++	++	++	++	++	++	++	++
Alkaloids	++	++	++	+	+++	+++	+	+
Flavonoids	++	++	++	+++	+++	++	++	+
Phenolics	++	+	++	++	++	+	++	++
Tannin	-	-	-	-	+	+	+	+
Glycoside	-	-	++	++	++	+	++	+
Saponin	++	++	+	+	++	+++	+	++
Steroids	-	-	-	-	-	-	-	-
Terpenoids	-	-	-	-	+++	++	+++	+++
Cardiac glycosides	+	+	++	+	+++	+++	++	+++

The total amount of phenols found in different extracts of AA leaf and stem estimated and presented in Figure 1A. The total phenolic found high quantity in ethanol extract of stem (167.54 mg GAE/ g extract) than the leaf extract (150.87 mg GAE/g extract) followed by ethyl acetate extract of stem showed high (133.2 mg GAE/g extract) than the leaf (128.5mg GAE/g extract). Hence, stem extracts have a better quantity of phenolic contents than leaf.

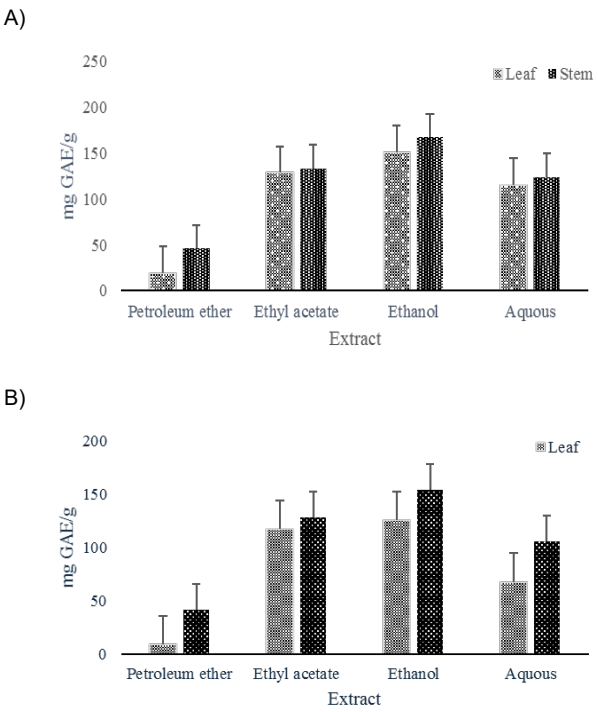

Figure 1 (A&B). Total phenolic and tannin content of A. alata leaf and stem extracts. mg/GAE/g – Gallic Acid Equivalents. Values are mean of triplicate determination (n=5) ± standard deviation, statistically significant at p<0.05 where a>b>c>d

Similarly, tannins contents also found higher in the ethanol extract of stem (154.15 mg GAE/g extract) than the ethanol extract of leaf (125.66 mg GAE/g extract). Also, stems have shown the maxi-mum amount of tannin presence revealed by ethyl acetate extract (128.49mg GAE/g extract) and the least amount observed in petroleum ether extracts of leaf (8.65 mg GAE/ g extract) (Figure 1B).

The presence of flavonoid contents in the leaf and stem of AA measured and presented in Figure 2. When comparing all the solvent extracts, the ethanol derived extracts of stem and leaf found appreciable quantity of flavonoids (88.14 mg RE/g extract and 80.97 mg RE/g extract respectively). In addition, the flavonoid content of the stem was higher than the leaf contents in most of the extracts. The minimum quantity observed in petroleum ether extract of stem (5.7 mg RE/g extract) and in aqueous extract of leaf (13.45 mg RE/g extract).

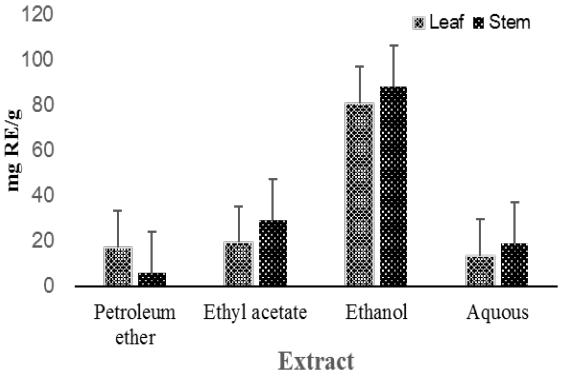

Figure 2: Flavonoid content of A. alata leaf and stem extracts. mg/GAE/g – Gallic Acid Equivalents. Values are mean of triplicate determination (n=5) ± standard deviation, statistically significant at p<0.05 where a>b>c>d

The TEAC (Trolox equivalents antioxidant capability) evaluated using an advanced ABTS+ radical decolonization assay, one of the most utilized methods for antioxidant capacity, which measures the ability of a compound to scavenge ABTS cation radicals. The results expressed as µg Trolox equivalents/g of extract. The results of ABTS cation radical scavenging activities of leaf and stem extracts of AA are shown in Table 3.

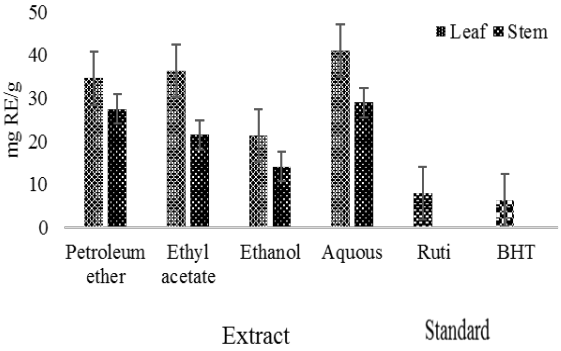

Figure 3: DPPH radical scavenging activity of A. alata leaf and stem extracts. mg/GAE/g – Gallic Acid Equivalents. Values are mean of tripli-cate determination (n=5) ± standard deviation, statistically significant at p<0.05 where a>b>c>d

The results have shown that the ethanol extracts of leaf and stem exhibited higher radical scavenging activities 43611.11 and 61284.72 µg TE/g extract respectively compared to other solvent extracts, whereas standard control rutin, which is natural antioxidant and synthetic antioxidant BHT have shown 68645.8 µg TE/g sample and 69236.1 µg TE/g extract respectively.

The DPPH radical scavenging activities of AA leaf and stem extracts shown in Figure 3. In this method, commercially available synthetic BHT and natural antioxidant rutin are used as standard controls. The concentration of the sample necessary to decrease initial concentration of DPPH by 50% (IC50) under the experimental condition was determined prior to the assays. All the solvent extracts subjected for analysis, in which, the ethanol extract of stem (14.15 µg/ml) and leaf (21.37 µg/ml) and ethyl acetate extract of stem (21.54 µg/ml) shown stronger IC50values for DPPH radical scavenging activities, when comparing other solvent extracts. However, the IC50value of aqueous extract of leaf (41.06 µg/ml) have shown less free radical scavenging activity. The IC50 value of standards (rutin and BHT) was 7.93 and 6.35 µg/ml respectively.

In addition to the above methods, the present study adopted riboflavin- NBT- light system assay. In this method, AA plant extracts have shown efficient scavenging activity against superoxide radicals. The quenching activity of ethanol extract of stem was 40.51% when compared to aqueous extracts of leaf and stem, which is 32.73% and 32.1% respectively. It seems ethanol and aqueous extracts have shown moderate free radical scavenging activity when compared to standard controls like synthetic and natural antioxidants (BHT and ru-tin) and shown superior radical inhibition. The percentages of inhibition of all the extracts presented in Figure 4. These outcomes suggest that the extracts of plant AA exhibited good scavenging properties against superoxide radicals.

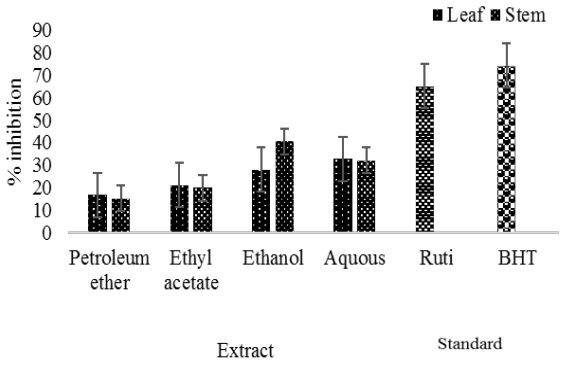

Figure 4: Superoxide radical scavenging activity of A. alata leaf and stem extracts

The principle of phosphomolybdenum method is based on the reduction of Mo (VI) to Mo (V) by the antioxidant compound and the formation of green phosphate/Mo (V) complex, which measured at its maximal absorption at 695 nm. The present study adopted this method to measure the total antioxidant ability of different solvent extracts of AA leaf and stem, which are shown in Figure 5. The ethanol extract of stem (255.66 mg AAE/g extract) have exhibited better antioxidant ability when compared to leaf (198.21 mg AAE/g extract), whereas lower antioxidant capacities were evident in petroleum ether extracts of both leaf and stem as they possessed only 58.59 and 67.4 mg AAE/g extracts respectively.

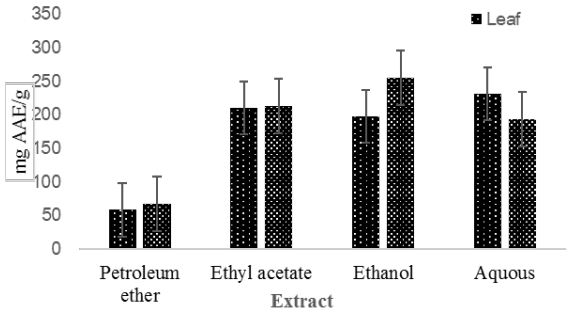

Figure 5: Phosphomolybdenum reduction assay of A. alata leaf and stem extracts. mg/AAE/g – Acetic Acid Equivalents. Values are mean of trip-licate determination (n=5) ± standard deviation, statistically significant at p<0.05 where a>b>c>d

The antimicrobial activity of AA tested against both bacterial and fungal strains. Among the bacterial strains used, Bacillus subtilis found to be inhibited more than the rest of the positive and negative strains. The positive bacterial strains showed the highest inhibition, and these results when compared to positive control, ampicillin. The ethanol extract of AA stem has shown more inhibition against Aspergillus fumigates than the other tested fungal strains. The leaf extract of AA also showed similar results and indicated that results were comparable with the standard, terbinafine.

Table 4: ABTS radical cation scavenging activity of A. alata leaf and stem extracts. TE – Trolox Equivalents, Values are mean of triplicate determination (n=3) ± standard deviation, statistically significant at p<0.05 where a>b>c>d in each column

Samples	Extracts	ABTS (µg TE/g extract)
Leaf	Petroleum ether	26319.44 ± 492
	Ethyl acetate	34375 ± 625
	Ethanol	**43611.11 ± 513**
	Aqueous	39722.22 ± 524
Stem	Petroleum ether	22048.61 ± 809
	Ethyl acetate	50798.61 ± 1634[d]
	Ethanol	**61284.72** ± 433[c]
	Aqueous	52881.94 ± 1294
Standard	Rutin	68645.8 ± 208[b]
	BHT	69236.1 ± 318[a]

Antibacterial activity of AA leaf and stem extracts derived using various solvents like petroleum ether, ethyl acetate, ethanol and aqueous extract

tested against ten human bacterial pathogens, which were both gram positive and gram negative.

Table 5: Antibacterial Activity of A. alata leaf and stem extracts. Values are mean of triplicate determination (n=3) ± standard deviation

(Gram Positive Bacteria) Zone of Inhibition (mm)						
Plant part	**Plant Extracts**	***Staphylococcus aureus***	***Bacillus cereus***	***Bacillus subtilis***	***Staphylococcus epidermis***	***Streptococcus Pyogenes***
Leaf	Petroleum ether	-	-	-	-	-
	Ethyl acetate	2.11 ± 0.41	2.35 ± 0.41	18.04 ± 0.20	-	3.01 ± 0.81
	Ethanol	16.05 ± 0.18	13.20 ± 0.18	17.12 ± 0.57	18.17 ± 0.13	13.06 ± 0.65
	Aqueous	11.14 ± 0.42	9.15 ± 0.40	16.14 ± 0.05	14.06 ± 0.91	10.40 ± 0.84
Stem	Petroleum ether	-	-	16.08 ± 0.11	-	-
	Ethyl acetate	-	9.30 ± 0.41	14.41 ± 0.57	3.51 ± 0.05	7.05 ± 0.90
	Ethanol	17.08 ± 0.30	12.71 ± 0.05	20.03 ± 0.3	19.47 ± 0.80	14.08 ± 0.84
	Aqueous	11.02 ± 0.81	11.40 ± 0.20	11.05 ± 0.71	10.34 ± 0.80	11.81 ± 0.41
Standard	Amphicillin (10µg)	23.07 ± 0.72	22.02 ± 0.07	23.06 ± 0.04	23.06 ± 0.03	22.07 ± 0.05
(Gram Negative Bacteria)						
Plant part	**Plant Extracts**	***Enterobacter cloacae***	***Escherichia coli***	***Klebsiella pneumoniae***	***Pseudomonas aeruginosa***	***Acinetobacterbaumannii***
Leaf	Petroleum ether	-	14.11±0.80	-	-	-
	Ethyl acetate	-	16.71±0.43	12.08±0.77	-	-
	Ethanol	16.03 ± 0.71	16.09±0.30	14.49±0.03	16.89±0.19	15.09 ± 0.80
	Aqueous	13.81 ± 0.30	13.47±0.61	9.1±0.35	10.49±0.08	11.79 ± 0.07
Stem	Petroleum ether	-	-	-	12.49±0.39	-
	Ethyl acetate	17.71 ± 0.15	16.01±0.42	-	4.11±0.72	4.05 ± 0.02
	Ethanol	18.47 ± 0.30	17.40±0.51	16.71±0.11	17.81±0.17	19.43 ± 0.11
	Aqueous	10.01 ± 0.11	10.19±0.37	10.90±0.14	13.04±0.80	14.04 ± 0.74
Standard	Amphicillin (10µg)	23.08 ± 0.10	20.19±0.01	19.30±0.40	20.01±0.06	23.05 ± 0.04

The antibacterial results of different extracts presented in Table 5 and Figure 6 (a-d). In general, the ethanol extract of the stem sample showed a significant zone of inhibition against Staphylococcus aureus (17.08 mm), Bacillus cereus (12.71 mm), Bacillus subtilis (20.03 mm), Staphylococcus epidermis (19.74 mm), Streptococcus Pyogenes (14.08 mm), Enterobacter cloacae (18.71 mm), Escherichia coli (17.40 mm), Klebsiella pneumoniae (16.71 mm), Pseudomonas aeruginosa (17.81 mm) and Acinetobacter baumannii (19.43 mm) at a concentration of 20 mg/ml. The zone of inhibition was better observed with the ethanol extract of AA stem; however, the inhibition declined for ethyl acetate, aqueous and chloroform solvent extracts. These results indicate that the stem extracts have shown better and promising antibacterial activity.

Table 6: Antifungal activity of A. alata leaf and stem extracts

Plant part	Plant Extracts	*Candida albicans*	*Aspergillus fumigatus*
Leaf	Petroleum ether	-	-
	Ethyl acetate	7.46 ± 0.32	13.43 ± 0.37
	Ethanol	16.25 ± 0.27	18.23 ± 0.32
	Aqueous	8.43 ± 0.65	14.36 ± 0.16
Stem	Petroleum ether	-	-
	Ethyl acetate	8.12 ± 0.26	13.26 ± 0.36
	Ethanol	16.26 ± 1.26	19.28 ± 0.26
	Aqueous	7.16 ± 0.08	16.23 ± 0.47
Standard	Terbinafine (10µg)	18.26 ± 0.56	21.06 ± 0.61

The present study further explores the antifungal activities using various extracts of AA. The antifungal results have revealed the inhibition increased linearly with the increase in concentration of the extracts (µg/ml) when compared to standard controls. Aspergillus fumigatus showed better performance than Candida albicans. The growth inhibition zone measured ranged from 21 mm for all the fungal strains used (Table 6 and Figure 6e).

Discussion

New drug leads sustainable development or nature derived pharmaceutical entities exploration is always a constant and progressive systemic approach to explore their therapeutic characteristics [95]. With the advent of many cutting-edge techniques and tools aid to reveal the structural and functional nature of many natural products which include herbal compounds [96]. At present, 25% of medicinal plant derivatives are in use either as an anticancer or as an anti-infective drug [97]. Although, phytochemicals provide a substantial outcome at the clinic [98], yet these drug leads well-described mechanisms of action is imperative for the sustainable pharmaceutical development to avoid any complexity as well as existing therapeutic difficulties if any.

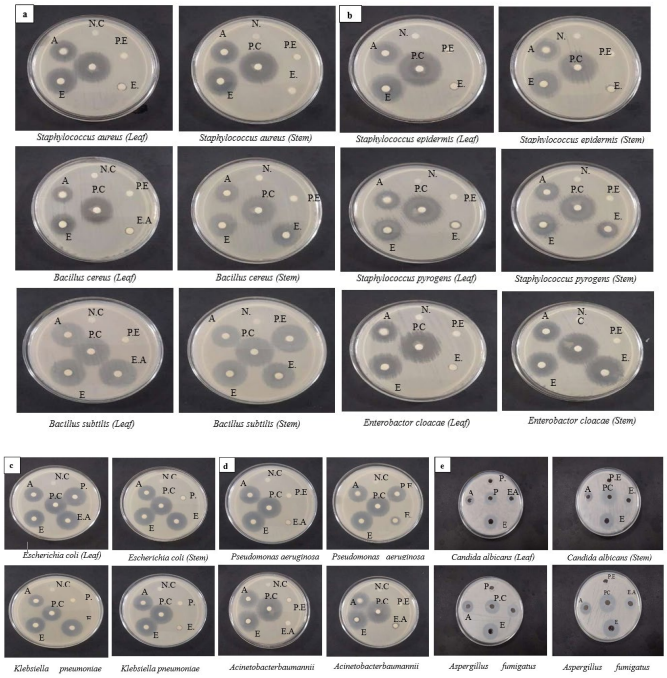

Figure 6(a-e): Antibacterial and antifungal activity of A. alata leaf and stem extracts

Despite the volume of scientific information available on pharmaceutical development in the modern era, the search continues towards the discovery of herbal medicine for the benefit of human beings [99]. In line with this, various ethnobotanical reports established that AA herbs have proven its efficacy against various diseases. The leaves and stem of AA used to isolate various extracts by using petroleum ether, ethyl acetate and ethanol and macerated using aqueous and finally concentrated by rotary evaporator. Amongst all the ex-tracts, ethanol derived exhibited maximum yield in all parts of the selected samples. The qualitative phytocompounds screening of AA revealed the presence of the following primary compounds such as amino acids, carbohydrates, and proteins both in leaf and stem extracts. The secondary bioactive principles such as alka-loids, flavonoid, phenolic, saponins, and cardiac

glycosides distributed differently in various parts of the AA. It also noted that the secondary metabolites that analyzed in the selected species have beneficial use in pharmaceutical industries [100]. The phytochemical investigations have shown the existence of phenolics as main metabolites, in agreement with earlier reports explored on various plant parts like roots, barks, fruits, leaves and stems of Andrographis species [101], [102], [103]. These results suggest that the phenolic compounds of AA have proven to be potent antioxidants.

In general, phenols are fragrant bioactive principles which give protection to the medicinal plants against stress and have antioxidant ability [104]. Phenol compounds are constant phenoxy radicals, interrupting oxidation reactions in various components in the cell [105] and play a precise role in scavenging ROS [106]. These compounds highly contribute to the antioxidant capacity which helps in maintaining health and protection from severe disorders [107]. The current report has shown the occurrence of higher phenol contents in selected samples of AA than in the Andrographis echioides [108]. To support this, Adiguna et al. [109] has reported Andrographis paniculata has total phenolic content of 30.68mg GAE/g. Therefore, the higher quantity of secondary bioactive principles exist in the AA are the direct indication for its higher anti-oxidative role, hence AA plant can be used as an alternative source to obtain a novel drug lead.

Tannins are organic compounds with bitter taste and exhibit strong astringent properties against inflammation and free radicals, microbes and protein precipitation. Previous studies [108], [109], [110] have worked on A. Paniculata (52.85 mg/g), and the results were comparable with AA (18.35 mg/g). The presence of tannin in the plant system contributes to the defense of the plant. Flavonoids are a diverse group of important naturally occurring phenols. In the food processing industry, flavonoids are employed to prevent heat or chemical-induced lipid peroxidation mechanism and chelating metallic and superoxide ions [111]. Medici-nal and non-nutritive plant derived flavonoids used as natural antioxidants in the food processing industry ow-ing to their ability to inhibit and scavenge ROS. [112]. Shilpam and Richa [113] have worked on A. paniculata (61.125 mg/ RE/g) and have reported that it has elevated levels of flavonoid. Comparatively the selected study species AA stem (88.14RE/g) and leaf (80.97 RE/g) contribute high amount of flavonoid content and show positive correlation among various study species.

The DPPH method is widely employed to assess the ability of the herbal extracts and foods to quench radical species or hydrogen donors to assess their activities against oxidative stress [114]. The present antioxidant results have shown that the ethanol extracts of AA leaf and stem showed higher free radical scavenging properties that may be due to the higher extractability and solubility of antioxidant compounds like phenols, and other metabolites found in AA extracts. The total antioxidant activity of AA may be owing to the presence of higher concentration of phenolic contents, which can directly contribute to the potential scavenging potential and nutraceutical activity [115], [116]. In line with this, Gurupriya and Cathrine [108] reported that bark extract of Andrographis echioides have exhibited stronger radical quenching activity. In line with this, Rajeshwari et al. [117] reported that A. paniculata also showed high DPPH radical scavenging activity. To sup-port the above, Shreya Reddy et al. [118] analyzed the scavenging activity of A. paniculata leaf, where the IC50 value ranged from 20-40 µg/mL. Besides, Kripasana and Xavier [119] has done comparative antioxidant assay on Acanthaceae family in different genus which exhibited high antioxidant capacity with an IC50 value Phlogacanthus pubinervius (77.83%), Adhatoda vasica (74.81%), Phlogacanthus curviflorus (94.20%), and Ru-ellia tuberosa (70.78%). These comparative reports on scavenging activities of Acanthaceae members have suggested that the radical species scavenging ability may be due to its higher phenolic contents [120], [121].

In addition to DPPH method, the present study employed ABTS assay as it is an important tool in determining the radical scavenging activity, since it is soluble nature both in aqueous as well as in organic solvents and not affected by any change in ionic strength of hydrogen donating compounds and of chain-breaking anti-oxidants like scavengers of both aqueous and lipid peroxyl radicals [122]. The results of AA ethanol extracts have shown the maximum radical scavenging activity both in leaf; 43611.11µM TE/g and stem; 61284.72µM TE/g respectively. Jeevanantham and Zahir Hussain [123] analyzed the radical scavenging activity of An-drographis echioides, which supported our results that the leaf ethanol extract depicted with 104.54 µg/mL TE/g. Similarly, Adiguna et al. [109] also performed ABTS radical scavenging assay using A. paniculata leaf, their results have shown the range from 0.25-84.13 µg/ml. When compared with previous reports with selected Andrographis species the current study revealed that the other members have shown less scavenging than AA.

The Phosphomolybdenum method is a simple evaluation test of a total antioxidant measure of plant de-rived compounds. The results of this quantitative antioxidant assay expressed as ascorbic acid equivalents (mg/g fruit extract). The basic principle behind this method is that the reduction of Mo (VI) to Mo (V) by the antioxidant agents and the subsequent formation of a green phosphate/Mo (V) complex with a maximal absorption at 695 nm [124]. In this regard, Eyazul et al. [125] have reported that the acetone extract of A. paniculata leaf shown higher antioxidant activity ranging from 0.05 to 0.50 mg AAE/g extract). To support this, Adiguna et al. [109] reported that A. paniculata and A. zeylanica ethanol leaf extract exhibit appreciable antioxidant potential in the range (53.9 - 65.9 mg AAE/g extract). The present study used various solvents for extraction of bioactive principles,

amongst, the ethanol extract revealed better antioxidant ability in comparison with various other solvent extracts. Thus, the antioxidant potential observed from various AA extracts are correlated well with its radical species quenching activity.

In addition, phytocompounds radical scavenging activity extended to superoxide (O−2) assessment. Superoxide is a reduced form of molecular oxygen and formed from mitochondrial electron transport systems [126]. Mitochondria chain reactions reduce oxygen to water. During this reaction, some electrons escape and directly react with oxygen and form superoxide. It is well established that superoxide plays a key role in the formation of other ROS like hydrogen peroxide, hydroxyl, or singlet oxygen in living systems [127]. To sup-port the above, the present results confirmed that various solvent derived extracts of AA have demonstrated good scavenging activity on superoxide radicals in a concentration dependent manner. Among those extracts tested, ethanol extracts of AA leaf (28.4%) and stem (40.51%) showed higher O−2 radical scavenging activity. To compare the scavenging activity of other members of this Andrographis species, unfortunately a less or on-ly sparse data available on O−2 scavenging properties. However, a report by Seval et al. [128] investigated Acanthus hirsutus, in which the results showed high radical scavenging activity suggest that amino acids like histidine, proline, alanine, and leucine are greatly to the scavenging of superoxide ions. This may be applicable to AA also as they possess high amino acids, which may contribute to high antioxidant and scavenging activities.

Among the various plant extracts used for the antibacterial assay ethanol extracts of AA showed a high degree of anti-bacterial activity with varied rate of inhibition against various pathogens subjected for this in-vestigation. The results have shown that the petroleum ether and ethyl acetate extracts showed less activity than ethanol extracts. Acinetobacter baumannii (19.43 mm), Bacillus cereus (12.71 mm), Bacillus subtilis (20.03 mm), Staphylococcus epidermis (19.74 mm), Streptococcus pyogenes (14.08 mm), Enterobacter cloacae (18.71 mm), Escherichia coli (17.40 mm), Klebsiella pneumoniae (16.71 mm), Pseudomonas aeruginosa (17.81 mm) and Staphylococcus aureus (17.08 mm). B. subtilis is a bacterial that produces subtilisin enzyme, which causes dermal allergic or hypersensitivity reactions [129]. K. pneumoniae plays a major role in causing several infections to human system some common infections are urinary tract infections, nosocomial infections, pneumonia, septicemias and soft tissue infection [130]. In some cases, K. pneumoniae leads to death in immune deficient patients. It is established that the pathogenic strains of E. coli cause urinary tract infections (UTI), neonatal meningitis and gastroenteritis, however, it rarely causes hemolytic uremic syndrome, peritonitis, mastitis, septicaemia and gram-negative pneumonia [131].

In addition, most of the food borne diseases caused by B. cereus produce vomiting, nausea, and diarrhea [132]. Antibacterial findings have suggested that the plant extracts effectively applied to the treatment of many microbial diseases initiated by different bacterial strains. Previous studies have investigated Andrographis species of viz. A. paniculata, A. lineate, A. echioides, A. nallamalayana, [101], [133], [134] amongst these A. paniculata showed good zone of inhibition against Klebsilla pneumoniae and Bacillus cereus. Besides, A. paniculata and A. echioides employed against several bacteria strains using well-diffusion method, in which, most of the bacterium was found to be sensitive against ethyl acetate and ethanol extract when compared to petroleum ether and hexane as these are low polar solvent. It is interesting to note that the present study results support the previous studies data that the gram-positive bacteria are more sensitive to plant extracts when compared to that of the gram-negative bacteria [135], [136].

It is known that fungal infections are not frequent like bacterial or viral infections, however, there is an increasing incidence of fungal infections and related resistance among humans in the last 2 decades [137], [138]. An effective antifungal therapy could play a significant role in healthcare; in this regard, the screening of traditional medicinal plants in search of new and novel antifungal agents are in progress [139], [140]. The search for novel antifungal agents relies on ethnobotanical information and ethno-pharmacological exploration [141], [142]. Candida albicans is the most common causative agent of mucosal infections and systemic infection and it is responsible for about 70% of fungal infections around the world [143]. Aspergillus fumigatus is the most ubiquitous fungal species in the environment [144]. A. fumigatus can cause a wide range of infections in both immunocompromised and immunocompetent individuals [145]. Hemalatha et al. [146] has reported that the ethyl acetate extract of leaves recorded highest anti-fungal activity against all the selected pathogens viz., Pythium aphanidermatum, Phytophthora capsici, Macrophomina phaseolina, Fusarium udum and Asper-gillus Niger and produced inhibition zone of diameter equal to the positive control. Macrophomina phaseolina and Aspergillus Niger found to be more sensitive to ethyl acetate extract. The existence of some of such secondary bioactive principles in a significant quantity in the investigated part of AA may have conferred the strong antifungal activity.

The present study highlights remarkable antioxidants and pharmacological properties of AA. Among various species of Andrographis, in which the versatile AA stood as the best source of various types of compounds with diverse chemical structure next to A. paniculata [147]. Moreover, the antioxidant activity of AA extracts has emerged as a promising food additive to substitute the current industry made synthetic antioxidants [66]. However, additional investigations are required for the isolation and

identification of specific bio-active principles in detail and their functional role by employing an appropriate in vitro and in vivo studies, which are very much required to understand their molecular mechanism of action, the resultant drug leads may be not only a food additive but also an affordable and consistent source of medicine for human welfare.

Conclusion

In summary, besides, A. paniculata, plant A. alata of Acanthaceae has been extensively employed as complimentary medicine in various Asian countries, which includes China, Malaysia, Srilanka and India, Various bioactive bitter principles have exerted several pharmacological and biological activities. Phytochemical screening discovered that phenols, flavonoids and tannins are the principal bioactive principles and derived from the aerial portion of this species. Various other compounds found are saponins, and cardiac glycosides distributed differently in various parts like leaf and stem possess varied biological activities. Although the re-sent study provided a strong insight into the phytochemistry, pharmacology of AA and its potential against various microbes and radical species. Nevertheless, additional investigations required us to fully understand the molecular mechanisms of various bioactive principles of this plant. Besides, numerous clinical and laboratory reports on the toxicity of AA plant extracts from various parts are also equally important to confirm their safe-ty and eligibility as source of novel drug leads. Based on the available evidence, the exact antimicrobial mechanisms of action of AA derived bioactive compounds are yet to be explored in detail. After careful con-sideration of the relevant supporting evidence, the present existing data suggest that bioactive principles de-rived AA can be of the potential agents that could serve the analogue for the synthetic drugs for the treatment of various human diseases in future.

Compliance with ethical requirements

As this is an evidence-based research article with the plant extracts, ethical approval is not required.

Availability of data and material

The authors confirm that the data supporting the findings of this study are available within the article.

Acknowledgements

Authors would like to thank Dr. M. Palanisamy, Scientist, Botanical Survey of India, Southern Regional Cen-tre, Coimbatore, 641003 for providing help on authenticating the plant species Andrographis alata.

References

1. De Pasquale F, Della Penna S, Snyder AZ, Marzetti L, Pizzella V, Romani GL, Corbetta M. A cortical core for dynamic integration of functional networks in the resting human brain. Neuron. 2012; 74(4): 753-764. https://doi.org/10.1016/j.neuron.2012.03.031 PMid:22632732 PMCid:PMC3361697

2. Sharma K, Assefa D, Ha S, Nile H, Le ET, Park SW. Temperature-dependent studies on the total phenolics, flavonoids, antioxidant activities, and sugar content in six onion varieties. Journal of Food and Drug Analy-sis.2015; 23(2): 243-252. https://doi.org/10.1016/j.jfda.2014.10.005 PMid:28911379 PMCid:PMC9351770

3. Das UN. Arachidonic acid in health and disease with focus on hypertension and diabetes mellitus: A re-view. Journal of Advanced Research. 2018; 11: 43-55. https://doi.org/10.1016/j.jare.2018.01.002 PMid:30034875 PMCid:PMC6052660

4. Seyed M, Elodemi M Photosensitizing Herbs as Potential Therapeutics: A Prospective Insights into their Mechanisms for the Development of Novel Drug Leads in War with Cancer and Other Human Diseases. Open Access Maced J Med Sci. 2024; 12(2): Ahead of Print. https://doi.org/10.3889/oamjms.2024.11883. https://doi.org/10.3889/oamjms.2024.11883

5. Seyed MA. A comprehensive review on Phyllanthus derived natural products as potential chemotherapeu-tic and immunomodulators for a wide range of human diseases. Biocatalysis and Agricultural Biotechnolo-gy. 2019; 17:529-37. https://doi.org/10.1016/j.bcab.2019.01.008

6. Ali-Seyed M, Vijayaraghavan K. Nutraceuticals for Wound Healing: A Special Focus on Chromolaena odo-rata as Guardian of Health with Broad Spectrum of Biological Activities. Nutraceuticals in Veterinary Med-icine. 2019; 541-562. DOI:10.1007/978-3-030-04624-8_36 https://doi.org/10.1007/978-3-030-04624-8_36

7. Omonkhelin, Nworgu A, Buniyamin I, Paul O, Owolabi J, Zuleikha N . Evaluation of the oxytocic activity of the ethanol extract of the roots of Alchornea cordifolia. International Journal of Green Pharmacy. 2010; 4(4):247-255. https://doi.org/10.4103/0973-8258.74133

8. Newman DJ, Cragg GM. Natural products as sources of new drugs over the nearly four decades from 01/1981 to 09/2019. Journal of Natural Products. 2020; 83(3): 770-803. https://doi.org/10.1021/acs.jnatprod.9b01285 PMid:32162523

9. Nair R, Chanda S. Antibacterial activity of Psoralea corylifolia L. seed and aerial parts with various extrac-tion methods. Research Journal of Microbiology. 2011; 6(2):124-131. Consumer No01064035103 https://doi.org/10.3923/jm.2011.124.131

10. Singh R, Sharma S, Sharma V. Comparative and quantitative analysis of antioxidant and scavenging poten-tial of Indigofera tinctoria Linn. extracts. Journal of Integrative Medicine. 2015; 13(4): 269-278. https://doi.org/10.1016/S2095-4964(15)60183-2 PMid:26165372

11. Ali-Seyed M, Vijayaraghavan K. Dengue virus infections and anti-dengue virus activities of Andrographis paniculata. Asian Pacific Journal of Tropical Medicine. 2020; 13(2): 49-55. https://doi.org/10.4103/1995-7645.275412

12. Krishnaraju AV, Rao TVN, Sundararajua D, Vanisreeb M, Tsayb HS, Subbarajua GV. Assessment of bioac-tivity of Indian medicinal plants using brine shrimp (Artemia salina) lethality assay. International Journal of Applied Science and Engineering. 2005; 2(1): 125-134.

13. Kong JM, Goh NK, Chia LS, Chia TF). Recent advances in traditional plant drugs and orchids. Acta Phar-macologica Sinica. 2003; 24(1):7-21.

14. World Health Organization (WHO). Regional Office for South-East Asia.: Traditional herbal remedies for primary health care.2020; WHO Regional Office for South-East Asia.https://iris.who.int/handle/10665/206024

15. Dias DA, Urban S, Roessner U. A Historical Overview of Natural Products in Drug Discovery. Metabolites. 2012; 2(2):303-336. https://doi.org/10.3390/metabo2020303 PMid:24957513 PMCid:PMC3901206

16. Katiyar C, Kanjilal S, Gupta A, Katiyar S. Drug discovery from plant sources: An integrated approach. An International Quarterly Journal of Research in Ayurveda. 2012; 33(1): 10-19. https://doi.org/10.4103/0974-8520.100295 PMid:23049178 PMCid:PMC3456845

17. Sasidharan S, Chen Y, Saravanan D, Sundram K, Latha L. Extraction, isolation and characterization of bio-active compounds from plants' extracts. African Journal of Traditional, Complementary and Alternative Medicine. 2011; 8(1): 1-10. https://doi.org/10.4314/ajtcam.v8i1.60483

18. Konan K, David N, Justin N, Lydie B, Souleymane M, Francis Y. Hepatoprotective and in vivo antioxidant activity of Olax subscorpioidea Oliv. (Olacaceae) and Distemonathus benthamianus Baill. (Caesalpinia-ceae). Pharmacognosy Magazine. 2015; 11(41): 111-116. https://doi.org/10.4103/0973-1296.149723 PMid:25709219 PMCid:PMC4329609

19. Atanasov AG, Zotchev SB, Dirsch VM, Supuran CCT. Natural products in drug discovery: advances and opportunities. Nature Review Drug Discovery. 2021; 20: 200-216. https://doi.org/10.1038/s41573-020-00114-z PMid:33510482 PMCid:PMC7841765

20. Chevalier G, Sinatra ST, Oschman JL, Sokal K, Sokal P. Earthing: health implications of reconnecting the human body to the earth's surface electrons. Journal of Environmental and Public Health. 2012; 20(12): 1-8. https://doi.org/10.1155/2012/291541 PMid:22291721 PMCid:PMC3265077

21. Houghton C, Casey D, Shaw D, Murphy K. Rigour in qualitative case-study research. Nurse Research-er.2013; 20 (4): 12-17. https://doi.org/10.7748/nr2013.03.20.4.12.e326 PMid:23520707

22. Gnanasekaran G, Murthy GVS. Epitypification of Andrographis Alata (Acanthaceae). Telopea: Journal of Plant Systematics. 2015; 18: 165-166. https://doi.org/10.7751/telopea8903. https://doi.org/10.7751/telopea8903

23. Balu, S. and C. Alagesaboopathi. Anti-inflammatory activities of some species of Andrographis Wall. (Ac-anthaceae). Ancient Science of Life. 1993; 13(1/2):180-184.

24. Balu AM, Rincón E, Luque R, Serrano L. Mechanochemical extraction of antioxidant phenolic compounds from Mediterranean and medicinal Laurus nobilis: A comparative study with other traditional and green novel techniques. Industrial Crops and Products. 2019; 141(7): 111805. https://doi.org/10.1016/j.indcrop.2019.111805

25. Yasothkumar N, Sankaralingam S. Antimicrobial Activity of Andrographis alata (Vahl) Nees. Journal of. Drug Delivery and Therapeutics. 2024; 11(3):41-42. https://doi.org/10.22270/jddt.v11i3.4713

26. Gamble JS. Flora of the presidency of Madras. Botanical Survey of India. Calcutta. 1956; 2:1049.

27. Damu AG, Jayaprakasam B, Gunasekar D. A new flavone 2'-glucoside from Andrographis alata. Jurnal of Asian Natural Product Research. 1998a; 1:133-138. https://doi.org/10.1080/10286029808039855 PMid:11261214

28. Damu AG, Jayaprakasam B, Rao KV, Gunasekar D. A flavones glycoside from Andrographis alata. Phyto-chemistry. 1998b; 49:1811-1813. https://doi.org/10.1016/S0031-9422(98)00342-2 PMid:11711108

29. Das B, Ramu R, Rao YK, Reddy MR, Harish H, Reddy VS, Ramakrishna KVS. Acylated 5, 7, 2', 6'-oxygenated flavone glycosides from Andrographis alata. Phytochemistry. 2006; 67:978-983. https://doi.org/10.1016/j.phytochem.2006.03.003 PMid:16624351

30. Dalawai D, Aware C, Jadhav JP, Murthy HN. RP-HPLC analysis of diterpene lactones in leaves and stem of different species of Andrographis. Natural Product Research. 2019; 1-4. https://doi.org/10.1080/14786419.2019.1662004 PMid:31496277

31. Kapil A, Koul IB, Banerjee SK, Gupta BD. Antihepatotoxic effects of major diterpenoid constituents of Andrographis paniculata. Biochemistry Pharmacology. 1993; 46:182-185. https://doi.org/10.1016/0006-2952(93)90364-3 PMid:8347130

32. Batkhuu JK, Hattori K, Takano F, Fushiya S, Oshiman K, Fjujimiya Y . Suppression of NO production in activated macrophages in vitro and ex vivo by neoandrographolide from Andrographis paniculata. Biology Pharmacology Bulletin. 2002; 25:1174-1174. https://doi.org/10.1248/bpb.25.1169 PMid:12230111

33. Murugan NA, Pandian CJ, Jeyakanthan J. Computational investigation on Andrographis paniculata phyto-chemicals to evaluate their potency against SARS-CoV-2 in comparision to known antiviral compounds in drug trails. Journal of Biomolecule Structure Dynamics. 2020; https://doi.org/10.1080/07391102.2020.1777901 PMid:32543978 PMCid:PMC7309306

34. Neeraja C, Hari Krishna P, Sudhakar Redd C, Giri CC, Rao KV, Reddy VD. Distribution of Androgrpahis species in different districts of AndhraPradesh. Proceedings National Academy of Sciences India, Section B: Biological Sciences. 2015; 85:601-606. https://doi.org/10.1007/s40011-014-0364-1

35. Okhuarobo A, Falodun JE, Erharuyi O, Imieje V, Falodun A, Langer P. Harnessing the medicinal properties of Andrographis paniculata for diseases and beyond: a review of its phytochemistry and pharmacology. Asian Pacific Journal of Tropical Diseases. 2014; 4(3):213-22. https://doi.org/10.1016/S2222-1808(14)60509-0

36. Abdallah EM, Alhatlani BY, de Paula Menezes R, Martins CHG. Back to Nature: Medicinal Plants as Promising Sources for Antibacterial Drugs in the Post-Antibiotic Era. Plants. 2023; 12(17):3077. https://doi.org/10.3390/plants12173077 PMid:37687324 PMCid:PMC10490416

37. Sivaperumal R, Ramya S, Veera AR, Rajasekaran C, Jayakumari R. Ethnopharmacological studies on the medicinal plants used by tribal inhabitants of Kottur Hills, Dharmapuri, Tamilnadu, India. Environ W Int J Sci Tech. 2010; 5(3): 57-64.

38. Sangameswaran B, Ilango K. Evaluation anti-hyperglycemic and anti-hyperlipidaemic activities of An-drographis lineata Nees on Streptozotocin induced diabetic rats. Jordan J Biol Sci. 2010; 3: 83-86.

39. Sangameswaran B, Vaijayanthimala P, Sakthipriya M. In vitro anti-arthritic activity of Cissus quadrangu-laris stem extract. Asian Journal of Pharmaceutical and Clinical Research. 2019; 12(1): 250-255.https://doi.org/10.22159/ajpcr.2019.v12i1.27353

40. Perumalsamy R, Ignacimuthu S. Antibacterial activity of some folklore medicinal plants used by tribals in Western Ghats of India. Journal of Ethnopharmacology. 2000; 69(3): 63-71. https://doi.org/10.1016/S0378-8741(98)00156-1 PMid:10661885

41. Sharma B, Sharma UK. Hepatoprotective activity of some indigenous plants. International Journal of Pharm Tech Research.2009; 4(3):1330-1334.

42. Dhiman M, Lakshika S, Abhishek D, Poonam D, Sharma MM. Traditional knowledge to contemporary medication in the treatment of infectious disease dengue: A review. Frontiers in Pharmacology. 2022; 13(2): 1-22. https://doi.org/10.3389/fphar.2022.750494 PMid:35359838 PMCid:PMC8963989

43. Hossain MS, Urbi Z, SuleA, Rahman KMH. Andrographis paniculata (Burm. f.) Wall. ex Nees: A review of ethnobotany, phytochemistry, and pharmacology. The Scientific World Journal. 2014; 20(4):1-28. https://doi.org/10.1155/2014/274905 PMid:25950015 PMCid:PMC4408759

44. Dai Y, Chen SR, Chai L, Zhao J, Wang Y, Wang,Y. Overview of pharmacological activities of Androgra-phis paniculata and its major compound andrographolide. Critical Review in Food Science and Nutrition. 2019; 59(1): S17-S29. https://doi.org/10.1080/10408398.2018.1501657 PMid:30040451

45. Lu J, Ma Y, Wu J, Huang H, Wang H, Wang X, Chen Z, Chen J, He H, Huang C. A review for the neuro-protective effects of andrographolide in the central nervous system. Biomedical Pharmacotherapy. 2019; 117:109078. https://doi.org/10.1016/j.biopha.2019.109078 PMid:31181444

46. Kadapatti SS, Murthy HN. Rapid plant regeneration, analysis of genetic fidelity, and neoandrographolide content of micropropagated plants of Andrographis alata (Vahl) Nees. J Genet Eng Biotechnol. 2021; 19(1):20. https://doi.org/10.1186/s43141-021-00122-5 PMid:33496903 PMCid:PMC7835653

47. Afzal S, Abdul Manap AS., Attiq A, Albokhadaim I, Kandeel M, Alhojaily SM. From imbalance to im-pairment: the central role of reactive oxygen species in oxidative stress-induced disorders and therapeutic exploration. Front Pharmacology. 2023; 14: 1269581. https://doi.org/10.3389/fphar.2023.1269581 PMid:37927596 PMCid:PMC10622810

48. Arteel G, Beier E. Environmental exposure as a risk-modifying factor in liver diseases known and un-knowns. Acta Pharmaceutica Sin B. 2021; 11(12): 3768-3778. https://doi.org/10.1016/j.apsb.2021.09.005 PMid:35024305 PMCid:PMC8727918

49. Bharathi VR, Kumudha VB, Jayashree Suseela L. Thirumal, M., Antioxidant and wound healing studies on different extracts of Stereospermum colais leaf. International Journal of Research in Pharmaceutical Sci-ences. 2018; 1(4): 435-439.

50. Kinnula VL, Regan EA, Mazur W, Meoni E, Toljamo T, Millar J, Vuopala K. Smoking and COPD increase sputum levels of extracellular superoxide dismutase. Free Radical Biology and Medicine. 2011; 51(3): 726-732. https://doi.org/10.1016/j.freeradbiomed.2011.05.008 PMid:21621610

51. Lobo A, Patil A, Phatak V, Chandra N. Free radicals, antioxidants and functional foods: Impact on human health. Pharmacognosy Review. 2010; 4(8): 118-126. https://doi.org/10.4103/0973-7847.70902 PMid:22228951 PMCid:PMC3249911

52. Sas A, Van der Mark M, Nijssen PCG, Vlaanderen J, Huss A, Mulleners WM, Laar T.V, Kromhout H, Ver-meulen, R. A case-control study of the protective effect of alcohol, coffee, and cigarette consumption on Parkinson disease risk: Time-Since-Cessation modifies the effect of tobacco smoking. PLoS ONE. 2014; 9(4): e95297. https://doi.org/10.1371/journal.pone.0095297 PMid:24788751 PMCid:PMC4005732

53. Singh A, Kumar A, Tewari D. An ethnobotanical survey of medicinal plants used in Terai Forest of western Nepal. Journal of Ethnobiology and Ethnomedicine. 2012; 8(1): 19-25. https://doi.org/10.1186/1746-4269-8-19 PMid:22591592 PMCid:PMC3473258

54. Hyun JK, Park J, Lee BK, Jeong GS, Lee CJ, Lee SH. Three-dimensional brain-on-a-chip with an intersti-tial level of flow and its application as an in vitro model of Alzheimer's disease. Laboratory on a Chip. 2015; 15(1): 141-150. https://doi.org/10.1039/C4LC00962B PMid:25317977

55. Tosun D, Dabbs K, Caplan R, Siddarth P, Toga A, Seidenberg M, Hermann B. Deformation-based mor-phometry of prospective neurodevelopmental changes in new onset pediatric epilepsy. Brain. 2011; 134(4): 1003-1014. https://doi.org/10.1093/brain/awr027 PMid:21398377 PMCid:PMC3069702

56. Kanter M, Sagiroglu T, Yagci MA, Sezer A, Erboga M. Protective effect of curcumin on cyclosporin A-induced endothelial dysfunction, antioxidant capacity, and oxidative damage. Toxicology and Industrial Health. 2012; 30(4): 316-327. https://doi.org/10.1177/0748233712456065 PMid:22903178

57. Lee SY, Kim YC, Choi I, Hur SJ, Kim GB. Effect of fermentation on the antioxidant activity in plant-based foods. Food Chemistry. 2014; 160(1): 346-356. https://doi.org/10.1016/j.foodchem.2014.03.112 PMid:24799248

58. Kim H, Vu TT, Tran VK, Le Dang Q, Nguyen HT, Kim H, Kim JC. In vitro antibacterial activity of select-ed medicinal plants traditionally used in Vietnam against human pathogenic bacteria. BMC Complemen-tary and Alternative Medicine. 2015; 16(1): 1-8. https://doi.org/10.1186/s12906-016-1007-2 PMid:26819218 PMCid:PMC4728873

59. Borneo R, Aranibar C, Pigni NB, Martínez ML, Aguirre A, Ribotta PD, Wunderlin DA. Influence of the extraction conditions on chia oil quality and partially defatted flour antioxidant properties. Journal of Food Science and Technology. 2022; 59(5): 1982-1993. https://doi.org/10.1007/s13197-021-05213-2 PMid:35531402 PMCid:PMC9046508

60. Vaou N, Stavropoulou E, Voidarou C, Tsigalou C, Bezirtzoglou E. Towards Advances in Medicinal Plant Antimicrobial Activity: A Review Study on Challenges and Future Perspectives. Microorganisms. 2021; 9(10):2041. https://doi.org/10.3390/microorganisms9102041 PMid:34683362 PMCid:PMC8541629

61. Cowan MM. Plant products as antimicrobial agents. Clin Microbiol Rev. 1999; 12(4):564-582. https://doi.org/10.1128/CMR.12.4.564 PMid:10515903 PMCid:PMC88925

62. Thawabteh A, Juma S, Bader M, Karaman D, Scrano L, Bufo SA, Karaman R. The biological activity of natural alkaloids against herbivores, cancerous cells and pathogens. Toxins. 2019; 11(11): 656-660. https://doi.org/10.3390/toxins11110656 PMid:31717922 PMCid:PMC6891610

63. Roy A, Saraf S. Ethnomedicinal approach in biological and chemical investigation of phytochemicals as antimicrobials with special emphasis to antibacterials. Pharmaceutical Reviews.2006; 4(1): 1-33.

64. Eloff J, Shuping DS. The use of plants to protect plants and food against fungal pathogens: a review. Afri-can Journal of Traditional Complementary and Alternative Medicines. 2017; 14(4): 120-127. https://doi.org/10.21010/ajtcam.v14i4.14 PMid:28638874 PMCid:PMC5471458

65. Gurib-Fakim A, Seebaluck R, Mahomoodally F. Medicinal plants from the genus Acalypha (Euphorbia-ceae): A review of their ethnopharmacology and phytochemistry. Journal of Ethnopharmacology. 2015; 159(3):137-157. https://doi.org/10.1016/j.jep.2014.10.040 PMid:25446604

66. Jayakumar T, Hsieh CY, Lee JJ, Sheu JR. Experimental and clinical pharmacology of Andrographis pa-niculata and its major bioactive phytoconstituent Andrographolide. Evidence-Based Complementary and Alternative Medicine. 2013; 1(2): 1-16. https://doi.org/10.1155/2013/846740 PMid:23634174 PMCid:PMC3619690

67. Bahmani M, Saki K, Shahsavari S, Rafieian-Kopaei M, Sepahv R, Adineh A. Identification of medicinal plants effective in infectious diseases in Urmia, northwest of Iran. Asian Pacific Journal of Tropical Bio-medicin. 2015; 5(10): 858-864. https://doi.org/10.1016/j.apjtb.2015.06.004

68. Bentham, G.; Hooker, J.D. (1862-1883). Genera plantarum ad exemplaria imprimis in herbariis kewensibus servata definita (3 vols.). London: L Reeve & Co. Retrieved 24 January 2014. https://doi.org/10.5962/bhl.title.747

69. Gangaram S, Naidoo Y, Dewir YH, El-Hendawy S. Phytochemicals and Biological Activities of Barleria (Acanthaceae). Plants (Basel). 2021; 11(1):82. https://doi.org/10.3390/plants11010082 PMid:35009086 PMCid:PMC8747396

70. Venmathi Maran BA, Iqbal M, Gangadaran P, Ahn BC, Rao PV, Shah MD. Hepatoprotective Potential of Malaysian Medicinal Plants: A Review on Phytochemicals, Oxidative Stress, and Antioxidant Mechanisms. Molecules. 2022; 27(5):1533. https://doi.org/10.3390/molecules27051533 PMid:35268634 PMCid:PMC8911738

71. Alam N, Nusrat JB, Rafiquzzaman M. Review on in vivo and in vitro methods evaluation of antioxidant activity. Saudi pharmaceutical journal. 2016; 21(2): 143-152. https://doi.org/10.1016/j.jsps.2012.05.002 PMid:24936134 PMCid:PMC4052538

72. Khan I, Jan SA, Shinwari ZK, Muhammad AI, Khan Y, Kumar T. Ethnobotany and medicinal uses of folk-lore medicinal plants belonging to family acanthaceae: An updated review. MOJ Biol Med. 2017; 1(2):34-38. https://doi.org/10.15406/mojbm.2017.01.00009

73. Intharuksa A, Arunotayanun W, Yooin W, Sirisa-Ard P. A Comprehensive Review of Andrographis panicu-lata (Burm. f.) Nees and Its Constituents as Potential Lead Compounds for COVID-19. Drug Discovery. Molecules. 2022; 27(14):4479. https://doi.org/10.3390/molecules27144479 PMid:35889352 PMCid:PMC9316804

74. Pandey A, Tripathi S. Concept of standardization, extraction, and pre-phytochemical screening strategies for herbal drug. J Pharmacogn Phytochem. 2014; 2:115-119.

75. Altemimi A, Lakhssassi N, Baharlouei A, Watson DG, Lightfoot DA. Phytochemicals: Extraction, isolation, and identification of bioactive compounds from plant extracts. Plants. 2017; 6:42. https://doi.org/10.3390/plants6040042 PMid:28937585 PMCid:PMC5750618

76. Sofowora A. The present status of knowledge of the plants used in traditional medicine in western Africa: A medical approach and a chemical evaluation. J Ethnopharmacol. 1980; 2:109-118. https://doi.org/10.1016/0378-8741(80)90004-5 PMid:7453197

77. Rungsung W, Ratha KK, Dutta S, Dixit AK, Hazra J. Secondary metabolites of plants in drugs discovery. World J Pharm Res. 2015; 4:604-613.

78. Liu R, Tang Y, Li X, Zhang B, Chen PX, Tsao R. Characterisation of phenolics, betanins and antioxidant activities in seeds of three Chenopodium quinoa Willd. genotypes. Food Chemistry. 2015; 166: 380-388. https://doi.org/10.1016/j.foodchem.2014.06.018 PMid:25053071

79. Sun Y, Li H, Deng Z, Liu R, Zhu H, Draves J, Marcone M, Tsao R. Characterization of phenolics, betacya-nins and antioxidant activities of the seed, leaf, sprout, flower and stalk extracts of three Amaranthus spe-cies. Journal of Food Composition and Analysis. 2015; 37: 75-81. https://doi.org/10.1016/j.jfca.2014.09.003

80. Hollman PCH, Cassidy A, Comte B, Heinonen M, Richelle M, Richling E, Vidry S. The biological rele-vance of direct antioxidant effects of polyphenols for cardiovascular health in humans is not established. The Journal of Nutrition. 2011; 141(5): 989S-1009S. https://doi.org/10.3945/jn.110.131490 PMid:21451125

81. Lim JCW, Chan TK, Ng DSW, Sagineedu SR, Stansias J, Wong WSF. Andrographolide and its analogues: versatile bioactive molecules for combating inflammation and cancer. Clin Exp Pharmacol Physiol. 2012; 39:300-310. https://doi.org/10.1111/j.1440-1681.2011.05633.x PMid:22017767

82. Ponphaiboon J, Krongrawa W, Aung WW, Chinatangkul N, Limmatvapirat S, Limmatvapirat C. Advances in Natural Product Extraction Techniques, Electrospun Fiber Fabrication, and the Integration of Experi-mental Design: A Comprehensive Review. Molecules. 2023; 28 (13):5163. https://doi.org/10.3390/molecules28135163 PMid:37446825 PMCid:PMC10343563

83. Trease GE, Evans WC. Textbook of pharmacognosy. 13th ed. London, UK; Toronto, Canada; Tokyo, Ja-pan: Bailiere Tindall 1989; 200-201.

84. Wallis TE. Textbook of pharmacognosy, Delhi, India: CBS Publishers and Distributors. 1989; 356-549.

85. Raaman N. Phytochemical Techniques. New India Publishing Agency. Jai Bharat Printing Press. New Del-hi.2006; 19-22. https://doi.org/10.59317/9789390083404

86. Beena P, Rajesh KJ, Arul B. Preliminary phytochemical screening of Cicer arietinum in folklore medicine for hepatoprotection. Journal of Innovative Pharmacology Biological Sciences. 2016; 3:153-159.

87. Abubakar AR, Haque M. Preparation of Medicinal Plants: Basic Extraction and Fractionation Procedures for Experimental Purposes. Journal of Pharmacology Bioallied Sciences. 2020; 12(1):1-10. https://doi.org/10.4103/jpbs.JPBS_175_19 PMid:32801594 PMCid:PMC7398001

88. Gursoy N, Sarikurkcu C, Cengiz M, Solak MH. Antioxidant activities, metal contents, total phenolics and flavonoids of seven Morchella species. Food Chemical Toxicology. 2009; 47(9): 2381-2388. https://doi.org/10.1016/j.fct.2009.06.032 PMid:19563856

89. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice EC. Antioxidant activity applying an im-proved ABTS radical cation decolourization assay. Free radical biology and Medicine. 1999; 26(1): 1231-1237. https://doi.org/10.1016/S0891-5849(98)00315-3 PMid:10381194

90. Beauchamp C, Fridovich I. Superoxide dismutase: improved assay and an assay applicable to polyacryla-mide gels. Analytical Biochemistry. 1971; 44(3): 276-287. https://doi.org/10.1016/0003-2697(71)90370-8 PMid:4943714

91. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitative of antioxidant capacity through the for-mation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. Analyti-cal Biochemistry. 1999; 269(1): 337-341. https://doi.org/10.1006/abio.1999.4019 PMid:10222007

92. Kotzé M, Eloff JN, Houghton PJ. Extraction of antibacterial compounds from Combretum microphyllum (Combretaceae). South African Journal of Botany. 2002; 68(1): 62-67. https://doi.org/10.1016/S0254-6299(16)30456-2

93. Tendencia EA. Disk diffusion method. In Laboratory manual of standardized methods for antimicrobial sensitivity tests for bacteria isolated from aquatic animals and environment. 2004; Chapter 2, 13-29.

94. Magaldi S, Mata S, Hartung C, Verde G, Deibis L, Roldín Y, Marcano C. In vitro susceptibility of 137 Can-dida sp. Isolates from HIV positive patients to several antifungal drugs. Mycopathologia. 2001; 149(2): 63-68. https://doi.org/10.1023/A:1007237711099 PMid:11265163

95. Lin KH, Yeh H, Lin SY, Yang CM, Tsai HJ, Tsai JJ, Chao PY. Antioxidant Activities of Methanol Extracts from Selected Taiwanese Herbaceous Plants. Journal of Food and Nutrition Research. 2014; 2 (8): 435-442. https://doi.org/10.12691/jfnr-2-8-2

96. Bauer A, Brönstrup M. Industrial natural product chemistry for drug discovery and development. Natural Product Reports. 2014; 31: 35-60. https://doi.org/10.1039/C3NP70058E PMid:24142193

97. Sala E, Guasch L, Iwaszkiewicz J, Mulero M, Salvadó MJ, Iade C. Identification of human IKK-2 inhibitors of natural origin (Part II): In silico prediction of IKK-2 inhibitors in natural extracts with known anti-inflammatory activity. European Journal of Medicinal Chemistry. 2011; 46: 6098-6103. https://doi.org/10.1016/j.ejmech.2011.09.022 PMid:22000921

98. Cosa P, Vlietinck AJ, Berghe DV, Maes L. Anti-infective potential of natural products: How to develop a stronger in vitro 'proof-of-concept. Journal of Ethnopharmacology. 2006; 106:290-302. https://doi.org/10.1016/j.jep.2006.04.003 PMid:16698208

99. Wachtel-Galor S, Benzie IFF. Herbal Medicine: An Introduction to Its History, Usage, Regulation, Current Trends, and Research Needs. In: Benzie IFF, Wachtel-Galor S, editors. Herbal Medicine: Biomolecular and Clinical Aspects. 2nd edition. Boca Raton (FL): CRC Press/Taylor & Francis; 2011. Chapter 1. Available from: https://www.ncbi.nlm.nih.gov/books/NBK92773/

100. Verma K, Singh S. Bio-pesticidal potential of the crude extract of Andrographis paniculata [Burm. F.) Wall ex. Nees] in the agricultural field: A review, Journal of Natural Pesticide Research. 2024; 8:2024. https://doi.org/10.1016/j.napere.2024.100076 https://doi.org/10.1016/j.napere.2024.100076

101. Polash SA, Saha T, Hossain MS, Sarker SR. Investigation of the phytochemicals, antioxidant, and an-timicrobial activity of the Andrographis paniculata leaf and stem extracts. Advances in Bioscience and Bio-technology. 2017; 8(3): 149-162. https://doi.org/10.4236/abb.2017.85012

102. Nagajothi S, Mekala P, Raja A, Raja MJ, Senthilkumar P. Andrographis paniculata: qualitative and quantitative phytochemical analysis. Journal of Pharmacognosy and Phytochemistry 2018; 7(4): 1251-1253.

103. Hariharan N, Vasan P. In vitro antioxidant activity of aqueous and alcoholic extracts of Andrographis paniculata whole plant powder by DPPH free radical scavenging assay. The Pharma Innovation Journal. 2023; 12(5): 3518-3521.

104. Tungmunnithum D, Thongboonyou A, Pholboon A, Yangsabai A. Flavonoids and other phenolic com-pounds from medicinal plants for pharmaceutical and medical aspects: an overview. Medicines. 2018; 5(3): 93. https://doi.org/10.3390/medicines5030093 PMid:30149600 PMCid:PMC6165118

105. Kaurinovic B, Vastag D. Flavonoids and phenolic acids as potential natural antioxidants. Antioxi-dants.2019; 2(1):1-14. https://doi.org/10.5772/intechopen.83731

106. Engwa GA. Free radicals and the role of plant phytochemicals as antioxidants against oxidative stress-related diseases. Phytochemicals - Source of Antioxidants and Role in Disease Prevention. 2018; 14(2): 61-73. https://doi.org/10.5772/intechopen.76719

107. Karimi E, Oskoueian E, Hendra R, Jaafar HZE. Evaluation of Crocus sativus L. stigma phenolic and flavonoid compounds and its antioxidant activity. Molecules. 2010; 15(9): 6244-6256. https://doi.org/10.3390/molecules15096244 PMid:20877220 PMCid:PMC6257777

108. Gurupriya S, Cathrine L. Qualitative and quantitative phytochemical analysis of Andrographis echi-oides leaves. International Journal of Life Science and Pharma Research. 2021; 11(1): 148-155. https://doi.org/10.22376/ijpbs/lpr.2021.11.1.P148-155

109. Adiguna SP, Panggabean JA, Swasono RT, Rahmawati SI., Izzati F, Bayu A, Putra MY, Formisano C, Giuseppina C. Evaluations of Andrographolide-rich fractions of Andrographis paniculate with enhanced po-tential antioxidant, anticancer, antihypertensive, and anti-Inflammatory activities. Plants.2023; 12(1): 1220. https://doi.org/10.3390/plants12061220 PMid:36986909 PMCid:PMC10052505

110. Hassan ZM, Manyelo TG, Selaledi L and Mabelebele M. The effects of tannins in monogastric animals with special reference to alternative feed ingredients. Molecules. 2020; 25(20): 4680. https://doi.org/10.3390/molecules25204680 PMid:33066367 PMCid:PMC7587385

111. Kumar S, Pandey K, Abhay K. Chemistry and biological activities of flavonoids: an overview. The Scientific World Journal. 2013; 20(2): 1-16. https://doi.org/10.1155/2013/162750 PMid:24470791 PMCid:PMC3891543

112. Kim SK, Kim YT, Byun HG, Nam KS, Joo DS, Shahidi F. Isolation and characterization of antioxida-tive peptides from gelatin hydrolysate of Alaska pollack skin. Journal of Agriculture and Food Chemistry. 2001; 49(4): 1984-1989. https://doi.org/10.1021/jf000494j PMid:11308357

113. Shilpam S, Raghuwanshi R. Evaluation of phytochemical, antioxidant and reducing activity in whole plant extract of Andrographis paniculata (Burm.f.) Wall. ex Nees. Bioscience Biotechnology Research Communications. 2020; 2(3): 1-16.

114. Garcia EJ, Oldoni TLC, Alencar SM, Loguercio AD, Grande RHM. Antioxidant activity by DPPH as-say of potential solutions to be applied on bleached teeth. Brazilian Dental Journal. 2012; 23(1): 22-27. https://doi.org/10.1590/S0103-64402012000100004 PMid:22460310

115. Rao NK. Anti-hyperglycemic and renal protective activities of Andrographis paniculata roots chloro-form extract. Iran Journal of Pharmacology Therapy. 2006; 5: 47-50.

116. Sani YN, Haque M, Suryati K, Mohd KW, Khan A. Isolation

and characterisation of andrographolide from Andrographis paniculata (Burm. F) wall. Ex nees and its total flavonoid effects from Kemaman. Ma-laysia. International Journal of Pharmacy Quality Assurances. 2017; 8: 119-124. https://doi.org/10.25258/ijpqa.v8i03.9573

117. Rajeswari VD, Eed EM, Elfasakhany A, Badruddin I, Kamangar S, Brindhadevi K. Green synthesis of titanium dioxide nanoparticles using Laurus nobilis (bay leaf): antioxidant and antimicrobial activities. Ap-plied Nanoscience. 2020; 13(3): 1477-1484. https://doi.org/10.1007/s13204-021-02065-2

118. Shreya Reddy CS, Ramalingam GD, Selvaraj J, Jothi Priya A. In vitro antioxidant and anti-diabetic analysis of Andrographis echioides and Andrographis paniculata ethanol extract. Bioinformation. 2022; 18(4):337-342. https://doi.org/10.6026/97320630018337 PMid:36909701 PMCid:PMC9997502

119. Kripasana K, Xavier J. Phytochemical analysis and antioxidant activity of leaf extracts of some select-ed plants of the family Acanthaceae. Plant Science Today. 2020; 7(2): 264-274. https://doi.org/10.14719/pst.2020.7.2.717

120. Sawadogo R, Lamien-Meda A, Lamien C, Martin K, Innocent G, Odile N. Phenolic Content and Anti-oxidant Activity of Six Acanthaceae from Burkina Faso. Journal of Biological Sciences. 2006; 6. 10.3923/jbs.2006.249:252. https://doi.org/10.3923/jbs.2006.249.252

121. Mussard E, Cesaro A, Lespessailles E, Legrain B, Berteina-Raboin S, Toumi H. Andrographolide, a Natural Antioxidant: An Update. Antioxidants (Basel). 2019; 8(12):571. https://doi.org/10.3390/antiox8120571 PMid:31756965 PMCid:PMC6943416

122. Rajakumar G, Thiruvengadam M, Mydhili G. Green approach for synthesis of zinc oxide nanoparticles from Andrographis paniculata leaf extract and evaluation of their antioxidant, anti-diabetic, and anti- in-flammatory activities. Bioprocess Biosystem Engineering. 2018; 41(1):21-30. https://doi.org/10.1007/s00449-017-1840-9 PMid:28916855

123. Jeevanantham K, Zahir Hussain A. Antimicrobial and antioxidant activity of Andrographis echioides (L.) Nees leaves extracts. International Journal of Biology, Pharmacy and Allied Sciences. 2021; 10(11): 01-15. https://doi.org/10.31032/IJBPAS/2021/10.11.1001

124. Kumaran A, Karunakaran, J. In vitro antioxidant activities of methanol extracts of five Phyllanthus species from India. LWT - Food Science and Technology. 2007; 40(2): 344-352. https://doi.org/10.1016/j.lwt.2005.09.011

125. Eyazul H, Ismet AJ, Hossain H, Nushrat JE, Ram PS, Syed MD, Iqbal M. Comparative studies on anti-oxidant potential of Andrographis paniculata grown in different places of Bangladesh. International Journal of Pharmaceutical and Phytopharmacological Research. 2013; 2(4): 252-258.

126. Jomova K, Raptova R, Alomar SY, Alwasel SH, Nepovimova E, Kuca K, Valko M. Reactive oxygen species, toxicity, oxidative stress, and antioxidants: chronic diseases and aging. Archives of Toxicology. 2023; 97(10):2499-2574. https://doi.org/10.1007/s00204-023-03562-9 PMid:37597078 PMCid:PMC10475008

127. Scialò F, Fernández-Ayala DJ, Sanz A. Role of mitochondrial reverse electron transport in ROS signal-ing potential roles in health and disease. Frontiers in Physiology. 2017; 8:1-7. https://doi.org/10.3389/fphys.2017.00428 PMid:28701960 PMCid:PMC5486155

128. Seval C, NazlıBökea B, İhsan Y, Süheyla K. A novel glycoside from Acanthus hirsutus (Acanthaceae). Natural Product Communications. 2020; 5(4): 563-566.

129. Yassin NA, Ahmad AM. Incidence and Resistotyping Profiles of Bacillus subtilis Isolated from Azadi Teaching Hospital in Duhok City, Iraq. Mater Sociomed. 2012; 24(3):194-197. https://doi.org/10.5455/msm.2012.24.194-197 PMid:23678324 PMCid:PMC3633405

130. Ashurst JV, Dawson A. Klebsiella Pneumonia. [Updated 2023 Jul 20]. In: StatPearls. Treasure Island (FL): StatPearls Publishing; 2024 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK519004/

131. Todar K. Bacterial resistance to antibiotics. The microbial world. Lectures in microbiology, University of Wisconsin-Madison. Journal of Biomedical Science and Engineering. 2012; 7(5): 5-10.

132. Kotiranta A, Lounatmaa K, Haapasalo M. Epidemiology and pathogenesis of Bacillus cereus infec-tions. Microbes and Infection. 2000; 2(2): 189-198. https://doi.org/10.1016/S1286-4579(00)00269-0 PMid:10742691

133. Kirthi AV, Ahmed IA, Sherif SH. Antibacterial activity of the extracts obtained from Eclipta alba. Hassk and Andrographis lineate against human pathogens. Journal of Pharmacy Research. 2010; 3(10): 2529-2532.

134. Elangovan K, Elumalai D, Anupriya S, Shenbhagaraman R, Kaleena PK, Murugesan K. Phyto mediat-ed biogenic synthesis of silver nanoparticles using leaf extract of Andrographis echioides and its bio- effi-cacy on anticancer and antibacterial activities. Journal of Photochemistry and Photobiology B: Biology. 2015; 151(8): 118-124. https://doi.org/10.1016/j.jphotobiol.2015.05.015 PMid:26233711

135. Taguri T, Tanaka T, Kouno I. Antibacterial spectrum of plant polyphenols and extracts depending upon hydroxyphenyl structure. Biological and Pharmaceutical Bulletin. 2006; 29(11): 2226-2235. https://doi.org/10.1248/bpb.29.2226 PMid:17077519

136. Padam BS, Vairappan CS, Tin HS, Abdullah MI, Chye FY. Effect of preparation and extraction param-eters of banana (Musa balbisiana cv. Saba) inflorescence on their antibacterial activities. Sains Malaysiana. 2015; 44(9): 1301-1307. https://doi.org/10.17576/jsm-2015-4409-12

137. Fostel JM, Lartey PA. Emerging novel antifungal agents. Drug Discovery Today.2000; 5(1): 25-32. https://doi.org/10.1016/S1359-6446(99)01430-0 PMid:10637546

138. Boussina A, Ramesh K, Arora H, Ratadiya P, Nemati S. Differentiation of Fungal, Viral, and Bacterial Sepsis using Multimodal Deep Learning. MedRxiv 2023; 11:2023.04.10.23288378. https://doi.org/10.1101/2023.04.10.23288378

139. Scorzoni L, de Paula E, Silva AC, Marcos CM, Assato PA, de Melo WC, de Oliveira HC, Costa-Orlandi CB, Mendes-Giannini MJ, Fusco-Almeida AM. Antifungal Therapy: New Advances in the Under-standing and Treatment of Mycosis. Front Microbiology. 2017; 8:36. https://doi.org/10.3389/fmicb.2017.00036 PMid:28167935 PMCid:PMC5253656

140. de Ullivarri FM, Sara A, Garcia-Gutierrez E, Cotter Paul D. Antifungal Peptides as Therapeutic Agents. Frontiers in Cellular and Infection Microbiology. 2020; 10: 2020. https://doi.org/10.3389/fcimb.2020.00105 PMid:32257965 PMCid:PMC7089922

141. Motsei ML, Lindsey KL, Staden J, Jäger AK. Screening of traditionally used South African plants for antifungal activity against

Candida albicans. Journal of Ethnopharmacology. 2003; 86(2-3): 235-241. https://doi.org/10.1016/S0378-8741(03)00082-5 PMid:12738093

142. Fortes TO, Alviano DS, Tupinambá G, Padrón TS, Antoniolli AR, Alviano CS, Seldin L. Production of an antimicrobial substance against Cryptococcus neoformans by Paenibacillus brasilensis Sa3 isolated from the rhizosphere of Kalanchoe brasiliensis. Microbiological Research. 2008; 163(2): 200-207. https://doi.org/10.1016/j.micres.2006.05.003 PMid:16790336

143. Morad HOJ, Wild AM, Wiehr S, Davies G, Maurer A, Pichler BJ, Thornton CR. Pre-clinical imaging of invasive candidiasis using ImmunoPET/MR. Frontiers in Microbiology. 2018; 9: 21-25. https://doi.org/10.3389/fmicb.2018.01996 PMid:30190717 PMCid:PMC6115526

144. Kwon-Chung KJ, Sugui JA. Aspergillus fumigatus - what makes the species a ubiquitous human fungal pathogen? PLoS Pathogens. 2013; 9(12): e1003743. https://doi.org/10.1371/journal.ppat.1003743 PMid:24348239 PMCid:PMC3857757

145. Denning DW, Chakrabarti A (2017). Pulmonary and sinus fungal diseases in non-immunocompromised patients. Lancet Infectious Diseases. 2017; 17(11): e357-e366. https://doi.org/10.1016/S1473-3099(17)30309-2 PMid:28774699

146. Hemalatha P, Sivakumar VR, Parimaladevi R, Tilak M. Studies on anti-fungal activity of leaf extracts of Andrographis echioides (L.) Nees. International Journal of Current Microbiology and Applied Scienc-es.2020; 9(10): 3853-3858. https://doi.org/10.20546/ijcmas.2020.910.443

147. Okhuarobo A, Falodun JE, Erharuyi O, Imieje V, Falodun A, Langer P. Harnessing the medicinal prop-erties of Andrographis paniculata for diseases and beyond: a review of its phytochemistry and pharmacolo-gy. Asian Pacific Journal Trop Diseases. 2014; 4(3):213-22. https://doi.org/10.1016/S2222-1808(14)60509-0

Abbreviations

AAE - Ascorbic Acid Equivalents
ABTS - 2, 2'-azinobis - 3-ethylbenzothiozoline-6-sulfonic acid
ALP - Alkaline phosphatase
ALT - Alanine aminotransferase
ANOVA - Analysis of variance
AOAC - Association of Official Analytical Chemists
AST - Aspartate aminotransferase
BCA - Bicinchoninic acid
BHA - Butylated hydroxyl anisole BHT- Butylated hydroxyl toluene
CAT - Catalase
CMC - Carboxy Methyl Cellulose
COX - Cyclooxygenase
CPCSEA - Committee for the Purpose of Control and Supervision of Experiments on Animals
DNS - 3, 5-Dinitrosalicylic acid
DPPH - 2, 2-Diphenyl-1-picryl-hydrazyl
EDTA - Ethylene DiamineTetraacetic Acid
FAO - Food and Agriculture Organization
FIC - Fractional Inhibitory Concentration
FRAP - Ferric Reducing Antioxidant Power
GAE - Gallic Acid Equivalents
GC-MS - Gas Chromatography–Mass Spectrometry
GPx - Glutathione peroxidase
GSH - Glutathione
HB - Haemoglobin
HPLC - High Performance Liquid Chromatography
IAEC - Institutional Animal Ethics Committee
IAA - Indole-3-acetic acid
IBA - Indole-3-butyric acid
IC50 - Half maximal inhibitory concentration
LPO - Lipid Peroxidation
NAA - 1-Naphthaleneacetic acid
NBT - NitroblueTetrazolium
NIST - National Institute of Standard Technology
OECD - Organization for Economic Co-operation and Development
PVPP - Polyvinyl Polypyrrolidine
RE - Rutin Equivalents
Rf - Retardation factor
ROS - Reactive Oxygen Species
Rpm - rotation per minute
SD - Standard Deviation
SDS - Sodium Dodecyl Sulphate
SGOT - Serum Glutamic Oxaloacetate Transaminase
SGPT - Serum Glutamic Pyruvate Transaminase
SOD - Superoxide Dismutase
TEAC - Trolox Equivalent Antioxidant Capacity
TPTZ - 2,4,6-Tripyridyl-s-Triazine
UV - Ultraviolet
WBC - White Blood Cells
WHO - World Health Organization