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In Vitro Antibacterial and Anti Cancerous Activity on Andrographis Paniculata Leaf Extract

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ABSTRACT

Andrographis paniculata, widely recognised as kalmeghor nilavembu, in traditional medicine, is a herb valued for its curative effects in the treatment of persistent health conditions. The study focuses on assessing the bioactive properties of *Andrographis Paniculata* leaf extracts, particularly its ability to act as an antioxidant and inhibit cancer-related activity.[1] The extracts were assessed for their total phenolic and flavonoid composition. Antimicrobial effectiveness was determined utilizing the well diffusion assay in accordance with the procedure outlined in [2].The ethanol extract displayed antibacterial activity across gram-positive and gram-negative bacterial strains. Furthermore, its antioxidant capability was also significant in vitro, as evidenced by assays including DPPH radical scavenging activity, reducing power assessment, and nitric oxide radical quenching. These antioxidant results were compared against standard compounds like D-ascorbic acid [3]. Importantly, the observed exhibited antiproliferative impact of the ethanol extract on against MCF-7 breast cancer cells underscores its potential therapeutic value in cancer treatment. The presence of compound andrographolide, in *A. paniculata*, confers antiproliferative properties, which may complement conventional chemotherapy strategies[8].

Keywords: *Andrographis paniculata, antioxidant, antimicrobial, antiproliferative, phenolic and flavonoid composition*

1. INTRODUCTION

Since ancient times, medicinal plants have been fundamental to maintaining health and treating diseases. Throughout human history, these natural resources served a crucial role in combating various illnesses. To date, around 80,000 plant species around the worldare recognized for their medicinal properties globally. Over the past two decades, interest in herbal medicine, partly due to their use in natural beauty products and traditional household treatments. Scientific research has increasingly focused on exploring the biological effects of these plants in disease management. Consequently, there is a rising trend toward encouraging the local cultivation of medicinal plants for healthcare purposes. The demand for pharmaceutical products, therapeutic drugs, herbal medicines, cosmetics, and personal care items is growing in both developing and developed countries. This surge is largely driven by the recognition that natural products tend to be safer, cause fewer side effects, and are more affordable and accessible than synthetic alternatives.[5]

Phytotherapy involving treatment with medicinal plants that possess antibacterial activity is a potential valuable alternative and potential source of pharmaceuticals. Plants are abundant with a variety of phytochemical constituents including alkaloids ,flavonoids,phenolic etc are fighting against the various diseases. Moreover, phytomedicines derived from plants also offer a lower cost source for medication and greater precision than chemotherapeutic drugs. *Andrographis paniculata,*called as 'King of Bitter, is a tiny, branched and erect herb belongs to the *Acanthaceae* family. It occurs in abundance in like Southeast Asia. It develop well in a variety of habitats like moist, shady places, plains, farms, seashores, and wet or dry lands. It possesses a broad spectrum of bioactive phytochemical like diterpenes, flavonoids and lactones .[6]

Andrographis paniculata is highly-regarded medicinal plant, notably recognized for its effectiveness in reducing fever and supporting the body's natural detoxification processes. It is regularly applied as a natural solution for avoiding and managing common cold infections. The therapeautic value has been formally acknowledged by Thailand Ministry of Public Health,which features it in the National Essential Herbal Medicines list. Recent findingssuggest that this plant contributes to a broad spectrum of health benefits. These include antioxidant, antidiarrheal, antibacterial, antifungal, hepatoprotective (liver-protecting),and anticancer activities [7].Various Taxonomical classification of *Andrographis paniculata* are shown in Table 1

Table 1 Taxonomical classification of *Andrographis paniculata*

Kingdom	**Plantae**
Class	Magnoliospida
Phylum	Tracheophyte
Divison	Angiosperm
Domain	Eukaryota
Family	Acanthaceae
Subclass	Gamopetalae
Genus	Andrographis
Tribe	Justticiae
Subfamily	Acanthoideae
Order	Personales
Species	*Paniculata(Burm.f)Nee*
Subtribe	*Andrographideae*

2. MATERIALS AND METHODS

Materials and methods section outlines the procedures,techniques used to collect and analyse data is given below:

2.1 Plant material Collection and extractionprocedure

Whole plant specimenof *Andrographis paniculata*specimensweregathered fromsurroundings locations and air-dried whole plant for 10-15 days. Grind into fine powder form and by using cold maceration extraction method, extract the ethanolic plant extract and stored in a refrigerator for further research.[8]

2.2 Phytochemical Analysis

Phytochemicals are the protective or disease preventive, non-nutritive phytocompounds of plants. They are constituents of the plant's defense system and also may provide health benefits to humans. Phytochemicals are biologically active secondary metabolites that plants produce through primary or secondary metabolism, serving important functions in plant growth and defense. Although not essential nutrients, they are extensively studied for their possible health benefits. Major groups are carotenoids and polyphenols, the latter including phenolic acids, flavonoids (e.g., anthocyanins, flavones, and flavanols), stilbenes, and lignans. Flavanols are subdivided into catechins, epicatechins, and proanthocyanidins. With an estimated 50,000 to 130,000 phytochemicals that have been identified, scientists investigate them by isolating compounds and examining their structures and biological activities in model systems in the laboratory, although identifying particular active compounds is still a challenge. [9-12]

Table 2 Phytochemical analysis

Phytochemical group	**Test method used**	**Result**
Alkaloids	Mayers, wagners, Drafendorfffs	Present(+)
Flavanoids	Shinoda, alkaline reagent test	Strongly present(++)
Phenolic compounds	Ferric chloride test	Strongly present(++)

Tannins	Gelatin and ferric chloride	Present(+)
Saponins	Froth test	Present(+)
Diterpenoids	Copper acetate test	Strongly present(++)
Glycosides	Keller-killiani test	Present(+)
Steroids	Liebermann-burchard test	Trace(+)
Terpenoids	Salkowski test	Strongly present(+)

2.3 Anti Oxidant Activity

The strong antioxidant potential of plant extract are mainly due to the abundance of biologically active compounds such polyphenols and flavonoids. To access the antioxidant capacity, of these extracts, several in vitro techniques are typically employed with DPPH and FRAP assays being among the most widely used. In particular, the antioxidant capacity of Andrographis paniculata has been assessed using the DPPH radical scavenging method. This assay determines how effectively a substance can neutralize free radicals. Research has shown that ethanol extracts of *A.paniculata* demonstrate significant antioxidant effects by scavenging DPPH radicals. With an IC50 value of 80µg/ml, the extract effectively inhibited nitric oxide radicals, although less efficiently than ascorbic acid, which recorded an IC50 of 40µg/ml .Furthermore, the ferric reducing antioxidant capacity of *A. paniculata* extract was estimated to be around 44% comparison to that of ascorbic acid [13-18].

2.4 Antimicrobial Activity

Agar Well diffusion wereused for analysis of bactericidal activities of ethanol extract *A.paniculata* [19].Among the test organisms were two Gram positive bacterial strains, *S.aureus, E.faecalis,*along with the two Gram negative bacterial strains *E.Coli, Klebsiella Phenumonia* and two fungi *Candida Albicans* and *Candida Tropicalis* are utilized for the antimicrobial activity. Molten Mueller Hinton Agar (MHA) media was filled into sterile petri plates and solidified. A suspension of bacteria (20-25 µl) was swabbed evenly on the solidified MHA plates.Sterile paper discswere immersed in solvents required and put on agar plates. 25 µl of leaf extract was filled into wells of agar plates .Plates were transferred to an incubator and held at the required conditions for 24 to 48 hours. Area of inhibition measured from well edge to zone in mm [20-22].

2.5 Anticancer Activities

Medicinal herbs have been applied for thousands of years in conventional folk medicine among Asian and African cultures. Most people in industrially developed countries also take products of plants these days for the health benefits of plants. For some countries, as per the World Health Organization (WHO), plant-based medication remains theirkey source of drugs. In developing countries, natural products are also being used with more frequency to serve as therapies. Interestingly, large number of components derived from and taxols[23]."Andrographolide" is a potent therapeutic anticancer pharmacophore, being the active constituent of A. paniculata. It strikes at the cancer cells both directly and indirectly. Good anti-tumorcounter to nasopharyngeal human epidermoid carcinomacell lines (KB) and lymphocytic leukemia celllines (P388) have been shown by the methanolic leaf extract of *A. paniculata* [24].

2.5.1. Determination of cell viability through MTT Assay

Using MTT assay, cell viability was measured based on the capacity on the living cells to reduce the yellow tetrazolium compound, MTT, to purple formazan by metabolically active cells. In this procedure, cells were cultured at a density of 1×10^4 cells per well in 96 well plates and treated with graded concentrations of ethanol-based extracts derived from Andrographis paniculata for a duration of 24 hours. Following the treatment, the existing culture medium was carefully removed and substituted with serum-free medium containing the MTT reagent. The plates were incubated at 37°C in a CO_2 atmosphere for 4 hours to allow them to convert MTT into formazan. After incubation, dimethyl sulfoxide (DMSO) was used to solubilize the formazan crystals, followed by absorbance measurement at 570 nm using a micro plate spectrophotometer [25-27].

3. COMMON MEDICINAL PLANTS AND ITS THERAPEAUTIC USES

3.1.Aloe Vera (*L.*) Burm.F

The following table 3 shows the phytochemical components, antioxidant, activity, antibacterial activity and anticancer activity of Aloe vera.

Table 3 Phytochemical components, antioxidant activity antibacterial activity and anticancer activity of Aloe vera

Medicinal plants	Phytochemical Components	Antioxident activity	Antibacterial Activity	Anticancer activity	References
Aloe vera (L.) BURM.F	Aloe vera extracts manifests the presence of flavonoids, phenols,tannin	High tannin and phenol conc. is good for the higher reducing power activity & higher flavonoid content is obtain stronger DPPH free radical scavenging activity	The plant derived of Aloe vera leaves demonstrated strong antimicrobial activity against Serratia marcescens bacteria, showing an inhibition zone measuring 13.67 ± 0.57 mm.	The ethanol-based extract from the leaves shows strong anticancer properties by effectively suppressing the proliferation of HepG2, HeLa, and A549 cancer cell lines.	[28 - 30]

3.2 Neem Leafs (*Azadirachta Indica*)

The following table 4 shows the phytochemical components, antioxidant, activity, antibacterial activity and anticancer activity of Neem leafs.

Table 4 Phytochemical components, antioxidant activity antibacterial activity and anticancer activity of Neem leafs

Medicinal plants	Phytochemical Constituent	Antioxidant activity	Antibacterial Activity	Anticancer Activity	Reference
Neem (*Azadirachta indica)*	The 50% ethanolic leaf extracts of neem exposed the presence of glycosides, phenols, tannin, proteins, flavonoids, alkaloids, triterpenoids, carbohydrates and alkaloids.	Phenolic and flavonoid content have been showed to contribute strong antioxidant activity	Leaf, oil and seed kernels extracts areexecute against some human fungi, such as *Trichophyton, Epidermophyton, Microsporum,Trichosporon,Geotricum&Candida* . Leaf oil ,seeds and bark contain astrong spectrum of antimicrobial action targeting against Gram-negative and Gram-positive microbes	Active constituents of *azadirachta indica* have been demonstrated to inhibit and medicinal benefits against oral cancer.	[31,32]

3.3.Turmeric (*Curcuma Longa*)

The following table 5 shows the phytochemical components, antioxidant, activity, antibacterial activity and anticancer activity of Turmeric.

Table 5 Phytochemical components, antioxidant, activity, antibacterial activity and anticancer activity of Turmeric

Medicinal plant	Phytochemical constituents	Antioxidant analysis	Antibacterial activity	Anticancer activity	References
Turmeric (*curcuma Longa)*	The turmeric extract exhibits diverse phytochemical constituents demonstrating the presence of coumarins, phenols, Steroids, Saponins, carbohydrates, flavanoids	The antioxidant activity of turmeric was determined via the DPPH assay, a method commonly used to measure free radical scavenging.when tested at a conc. of 100 µl/ml, turmeric essential oil showed a high level of efficacy, inhibiting free radicals by 79.8%, which highlights its strong antioxidant capability.	The essential oil demonstrated significant antibacterial activity, showing the greatest inhibition zone against Vibrio cholerae.Effective against various gram positive and gram negative microbial species	Plant extracts exhibit*in-vitro*toxicity to cells against HeLa cell lines and plants were found to be extremely powerful against various cancerous cells.	[33-35]

3.4. Ginger (*Curcuma Longa*)

The following table 6 shows the phytochemical components, antioxidant, activity, antibacterial activity and anticancer activity of Ginger.

Table 6 Phytochemical components, antioxidant, activity, antibacterial activity and anticancer activity of Ginger

Medicinal plant	Phytochemical constituents	Antioxidant analysis	Antibacterial Activity	Anticancer activity	References
Ginger (*zingiber officinale*)	The qualitative Assessment of Phytochemicalsin rhizome extracts indicated theappearance of reducing sugars, saponins,tannins,and glycosides	Presence of Phosphomolybdenum shows good antioxidant activity	Ethanolic extracts of Zingiber officinale (ginger) exhibit capable antibacterial performancetowards Staphylococcus aureus and Escherichia coli, while aqueous and acetonic extracts show minimal to no inhibitory effects	MTT assay results on ginger extract Exerted Differential cytotoxic effects on breast cancer cell lines.	[36,37,38]

3.5.Tulsi (*Ocimum Sanctum)*

The following table 7 shows the phytochemical components, antioxidant, activity, antibacterial activity and anticancer activity of Tulsi.

Table 7 Phytochemical components, antioxidant, activity, antibacterial activity and anticancer activity of Tulsi

Medicinal Plants	Phytochemical Constituent	Antioxidant activity	Antibacterial Activity	Anti cancer activity	References
Tulsi (*Ocimum sanctum*)	The ethanol leaf extract phytochemical screening detected the presence of anthraquinones, phenolic, steroids, saponins, aminoacids, proteins, tannin, flavonoids, triterpenoids.	Phenolics are act as strong efficiency on free radical scavengers and exhibit strong antioxidant activity	The ethanol extract of tulsi leaves indicates the disinfectant activity against pathogens	The extract confirmed anti-tumor properties by targeting the oral cancer cell line (Ca9-22). The identification phytochemicals found in the leaf extract suggests its potential effectiveness in combating oral cancer.	[39 - 41]

4. RESULTS AND DISCUSSION

The ethanolic extracts of *Andrographis paniculata* found to contain a various functional biologically active constituents such as alkaloids, flavonoids, saponins, reducing sugars, phenols,tannins steroids, phytosterols, cardiac glycosides, proteins and flavonoids Among these, several constituents were found to be present in significant quantities [42].Assessment of the ethanolic extracts antioxidant effect was conducted via the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging method, which revealed notable antioxidant capacity, with results closely aligning with standardascorbic acid. As the increase in extract concetration led to a proportional enhancement in DPPH inhibition.Similarly, the FRAP (Ferric Reducing Antioxidant Power) assessment indicated a substantial antioxidant efficacy in the ethanolic extract, which was nearly equivalent to the standard reference. A higher concentration of the extract corresponded to increased antioxidant activity [43].Furthermore, the ethanolic leaf extract of *Andrographis paniculata* was assessed for its potential anticancer activity targeting the MCF-7 humanbreast cancer cell line[44].

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