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Research

Evaluation Of Invitro Anti-Microbial And Anti-Oxidant Activities Of Plant Extract Of *Andrographis Paniculata*

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Abstract

Leaf extracts from *Andrographis Paniculata* are becoming more and more important due to their diverse biological and pharmacological characteristics. We now report on the anti-microbial and anti-oxidant investigation of leaf extracts from *Andrographis Paniculata*, continuing our work on diverse extracts. The leaves were gathered, dried, and ground into a coarse powder before being extracted over a 72-hour period using a Soxhlet apparatus with an aqueous and organic solvent. Following extraction, the leftovers were gathered, dried, and used for early research on compounds like carbohydrates, terpenoids, saponins, amino acids, alkaloids, and flavonoids, among others. After that, it is employed in research on antimicrobials. The extracts were subjected to antimicrobial study. During the antimicrobial study all the different concentration solvent were found to possess moderate antibacterial and antioxidant activity.

Keywords: *Andrographis Paniculata,* anti microbial, antioxidant, Herbal medicine, Plant extract.

INTRODUCTION

Numerous herbal plants are available in complementary and alternative medicine, and they may hold the key to solving the behind illnesses in humans. According to a World Health Organization (WHO) assessment, traditional plants are the primary source of health care for 80% of the population in under developed nations. [1,2] Natural remedies like herbal plant extracts (used in Ayurveda as described in the Charaka Samhita and Susruta Samhita or other traditional medical practices), plant-derived compounds (also known as phytoconstituents), extracts of particular plant parts (roots, stem, bark, flowers, fruits, and seeds), dietary supplements, and

nutraceuticals have a wide range of applications in the treatment of illnesses ranging from common to uncommon infectious and non-infectious diseases. Reports state that 25% of routinely used medications contain plant- derived chemicals.[3] Man has been familiar with plants since the dawn of time and has employed them in numerous ways throughout history. Primitive man developed to identify plants that were suitable for certain purposes in order to find sustenance and successfully deal with human difficulty.[4] Those with definite pharmacological effect for medical purposes, as the bond between man and plants grew, numerous plants began to be employed as remedies. As medical understanding about diseases continued to advance at an accelerated rate, so did the number of new medications made from plants India has sometimes been referred to as the Medicinal Garden of the globe since nature has gifted our nation with an amazing wealth of medicinal plants. Indian medical texts that discuss the use of plants.[5] Throughout the world, people depend on herbal cures and complementary medical treatments, and in the past, most pharmaceuticals original sources were frequently herbs. The plant world has made available an infinite supply of herbal teas, syrups, infusions, ointments, liniments, and powders that are made from medicinal plants that were first utilized in their rawest forms.

A burial site found in an underground location in northern Iraq that dates back around 60,000 years shows evidence of the usage of herbal treatments. An examination of the soil near the human bones found unusually large amounts of plant pollen from eight different species.[6,7] Seven of them are still used in the herbal world as medicinal herbs. The active components of numerous species have been identified and studied thanks to the advancement of chemistry and Western medicine. Because of this, researchers continue to look for and study obscure plants and to preserve those whose medicinal qualities have proven essential in the fight against disease.[8] A significant number of the commercial drugs used today for the treatment of heart disease, high blood pressure, and diabetes are still derived from herbs and ailments such as asthma, discomfort, and blood pressure.[9,10] For Example, ephedra is a herb that has been used for more than 2000 years in traditional Chinese medicine to treat asthma and other respiratory issues. The active component of ephedra, ephedrine, is utilized in commercial pharmaceutical preparations to treat respiratory issues including asthma.

A crucial component of the current research initiatives targeted at unlocking the anti-bacterial and anti-oxidant activity for plant phytochemical analysis is the development of quick and precise analytical methods as well as the creation of pure analytical and experimental standards of phytochemicals effects in the systems of plants and animals. These substances are frequently found in the plants themselves, relatively small amounts. They might only build up in specific situations, such the development of reproductive organs, or in response to particular kinds of stress. To as certain how these substances affect health, precise analytical techniques must be used in tandem with thorough biological research. As a result, research and development pertaining to therapeutic plants have gained significant importance. Lead molecules have been sourced from plants for a variety of disease.

Herbal medications are in high demand in both developed and developing nations due to their wide range of biological activities, therapeutic properties, higher safety margins, and lower costs. A significant portion of people in poor nations continue to use traditional medicine. Pharmacological screening and investigation of the chemical components of plants may give us the foundation for generating leads for the creation of novel drugs.

PLANT PROFILE

Synonyms: Creat or Green chiretta

Family: Acanthaceae

Fig 1: ***Andrographis paniculata***

Vernacular Names
Arabic: Quasabhuva
Chinese: Chuan Xin Lian
English: The Creat, King of Bitters
French : Chirette verte, Roi des amers
Gujarati : Kariyatu
Hindi: Kirayat, Kalpanath,
Japanese: Senshinren
Kannada : Nelaberu
Malayalam Nelavepu, Kiriyattu
Tamil : Siriya Nangai
Telugu : Nilavembu

Taxonomical Classification
Kingdom : Plantae, Plants;
Subkingdom : Tracheobionta, Vascular plants;
Super division : Spermatophyta, Seed plants;
Division : Angiosperma
Class : Dicotyledonae
Sub class : Gamopetalae
Series : Bicarpellatae
Order : Personales
Tribe : Justicieae
Family : Acanthaceae
Genus : Andrographis
Species : paniculate

Collection and authentication of plant

The plant was collected from the surrounding areas of Salem district, Tamilnadu, India and the plant was identified and authenticated at institute of Vivekanandha College of Arts and Science For Women College, Tiruchengode, Namakkal.

Extraction Procedure

The leaves of "***Andrographis Paniculata***" was dried under shade and then made in to a coarse powder with a mechanical grinder. The powder was passed through sieve No: 40 and stored in an air tight container for further use.

1) Preparation of petroleum ether extract

The dried powder material of leaves (250gm) was first extracted with petroleum ether (60-80º) in a Soxhlet apparatus and after complete extraction(48hrs), the solvent was removed by distillation under reduced pressure and resulting semi solid mass was vacuum dried using rotary flash evaporator to yield (5.0 % w/w) a solid residue.[26]

2) Preparation of ethanolic extract

After the extraction with petroleum ether the same plant material was dried and again extracted with ethanol (99.9 %v/v) in Soxhlet apparatus and after complete extraction (48hrs) the solvent was removed by distillation under reduced pressure and resulting semisolid mass was vacuum dried using rotary flash evaporator to yield (12.0 %w/w) a solid residue.

3) Preparation of aqueous extract

The aqueous extract was obtained by macerating 200gm of powdered plant material with 500 ml of distilled water (72hrs). The contents of the flask were mixed properly by gentle shaking by hand. Then the mixture was filtered and the filtrate was evaporated to dryness. The dried extract was stored in desiccators. The yield was about 15% w/w. All the three extracts were subjected to various chemical tests to detect the presence of different phytoconstituents.

Table 1: Anti-bacterial activity of "*Andrographis paniculata*" active against selective human pathogens.

Plant parts of *Andrographis paniculata*	Solvent used	Zone of inhibition (mm) of human pathogens			
		***Staphylococcus* sp.**	***E. Coli***	***Salmonella Typhi***	***Pseudomonas* sp.**
Root	Ethanol	11.3	16.7	15.2	11.2
	Acetone	12.7	17.3	18.7	14.3

	Methanol	13.2	22.8	18.7	13.8
	Water	10.3	14.7	11.7	11.1
	Ethanol	8.7	11.2	23.3	9.1
Stem	Acetone	13.4	17.6	19.4	9.2
	Methanol	16.6	29.2	18.8	19.6
	Water	10.2	10.3	13.3	7.5
	Ethanol	9.2	19.4	25.2	18.9
Leaf	Acetone	14.9	25.2	28.6	21.5
	Methanol	18.4	32.8	24.7	24.2
	Water	8.9	11.9	11.9	16.1

Phytochemical Screening Of "***Andrographis Paniculata***"

The plant extract was subjected to preliminary phytochemical screening for the detection of various plant constituents present. The term qualitative analysis refers to establishing and proving the identity of a substance. The active ingredients, after isolation, can be incorporated into the modern medicine for the development of newer formulation for therapeutic ailments. Qualitative phytochemical analysis The petroleum ether extract (PEEMC), ethanolic extract (EEMC) and aqueous extract of "***Andrographis paniculata***" were subjected to qualitative tests for the detection of various plant constituents.

Table 2: Phytochemical Evaluation test and extraction.

Phytoconstituents	Type of extracts		
	Petroleum Ether	Ethanol (99.9% v/v)	Aqueous
Carbohydrate	Present	Present	Present
Glycosides	Present	Present	Absent
Alkaloids	Present	Present	Present
Phytosterol and Steroids	Present	Present	Absent
Protein & Amino Acid	Present	Present	Present
Tannins	Present	Present	Absent
Flavonoids	Present	Present	Present
Saponins	Present	Present	Present
Terpenoids	Present	Present	Present

BIOLOGICAL EVALUVATION
IN VITRO ANTI-MICROBIAL SCREENING

The synthesized compounds were subjected to antimicrobial screening by disc diffusion method for zone of inhibition.

MATERIAL

Microorganisms used: Staphylococcus aureus (gram positive)
Bacterial strains: E-coli (gram negative)
Drugs (STD): Cefixime
Solvent (control): Distilled water and Ethanol
Solvent (control): Distilled water and Ethanol
Requirements: Nutrient broth/ sabourd dextrose agar
Petri dishes: Standardized culture of test organisms
Sterile pipettes
Test drug Standard drug

ANTIBACTERIAL STUDY
Preparation of inoculums

The inoculums for the experiment were prepared in fresh nutrient broth from the preserved slant culture. The turbidity of the culture can be adjusted by the addition of broth or sterile saline (if it is excessive) or by further incubation to get the required turbidity, and the newly prepared inoculums were standardized by adjusting the

turbidity of the culture to that of McFarland standards.

Preparation of sterile swabs

Cotton wool swab on wooden applicator or plastics were prepared and sterilized by autoclaving or by heat (only for the wooden swabs). It was sterilized by packing the swabs in culture tubes, papers or tins etc.

Sterilization of forceps

Forceps can be sterilized by dipping in alcohol and burning off the alcohol.

Experiment

The standardized inoculums is inoculated in the sterilized plates prepared earlier (aseptically) by dipping a sterile in the inoculums removing the excess of inoculums by passing and rotating the swab firmly against the side of the culture tube above the level of the liquid and finally streaking the swab all over the surface of the medium 3 times rotating the plate through an angle of 60º after each application. Finally pass the swab round the edge of the agar surface. Leave the inoculums to dry at room temperature with the lid closed. Each Petri dish is divided into 4 parts. Three compartments were loaded with sample as (100,200,300µg) and remaining compartment with standard Cefixime disc (10µg) with the help of sterile forceps. After that Petri dishes are placed in the refrigerator at 4ºC at room temperature for 1hour for diffusion. Incubate at 37ºC for 24 hours. Observe the zone of inhibition produced by different samples. Measure it using a scale and record the average of two diameters of each zone of inhibition.

ANTIOXIDANT SCREENING

DPPH **(α-diphenyl-β- picrylhydrazyl)** Free radical screening

Reagent preparation

0.1M DPPH solution was prepared by dissolving 2mg of DPPH in 20ml of ethanol.

Working procedure

Different volumes (10 - 50µl) of plant extracts were made up to 100µl with ethanol and 2.96ml DPPH (0.1mM) solution was added. The reaction mixture was incubated in dark condition at room temperature for 30 min.

After 30 min, the absorbance of the mixture was read at 517 nm. 3ml of DPPH was taken as control. The % radical scavenging activity of the plant extracts was calculated using the following formula, Where, RSA is the Radical Scavenging Activity; Abs control is the absorbance of DPPH radical + ethanol; Abs sample is the absorbance of DPPH radical + plant extract.

ANTIBACTERIAL STUDY

Table 3: Zone of inhibition (mm)

Compounds (µg/ml)	**Gram positive**	**Gram negative**
Aqueous (µg/ml)	**Staphylococcus**	**E-coli**
100µg	10mm	11mm
200µg	12mm	13mm
300µg	16mm	14mm
Standard drug Cefixime (10µg disc)	22mm	18mm
solvent control (distilled water)	-	-

(-) indicates no zone of inhibition

ANTIOXIDANT SCREENING

DPPH (α-diphenyl-β- picrylhydrazyl) Free radical screening

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Table 4: Ethanol extract of *Andrographis Paniculata*

S NO	Concentration(ml)	DPPH%
1	1ml	3.3
2	2ml	8.3
3	3ml	13.3
4	4ml	20
5	5ml	25

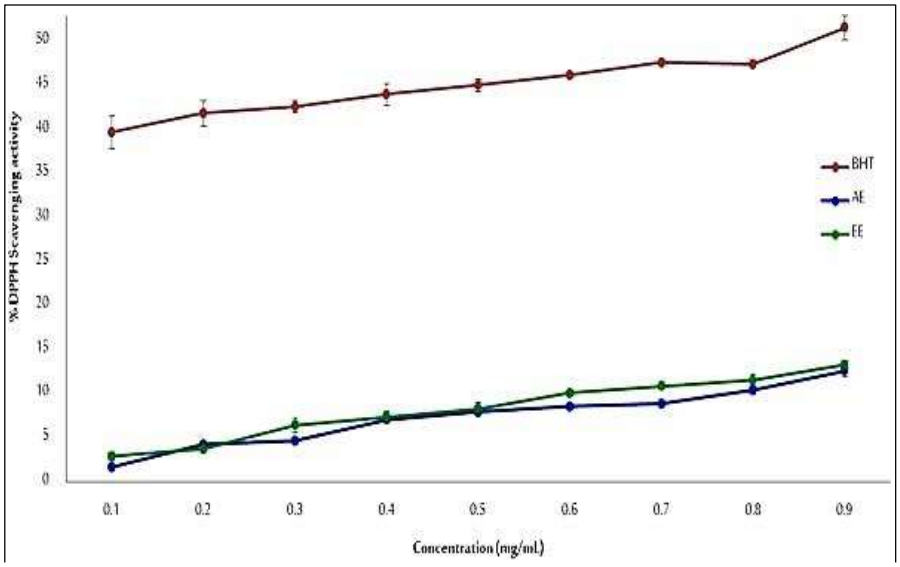


Fig 2: Total antioxidant activity of aqueous and ethanolic extracts of "*A. paniculate*" in comparison with BHT

RESULT AND DISCUSSION

Antibacterial screening

Using the Disc Diffusion Method, the aqueous and organic extracts of ***Andrographis Paniculata*****,** leaves were tested for their ability to inhibit the growth of both Gram-positive and Gram-negative bacteria, including *E. **coli*** and *staphylococcus aureus*. Every chemical was tested for its antibacterial activity against every type of bacteria at concentrations of 100µg/ml, 200µg/ml, and 300µg/ml. The aqueous extract was prepared using distilled water, while the organic extract was prepared using ethanol. Cefixime, the normal medication, and solvent control were kept for the investigation.

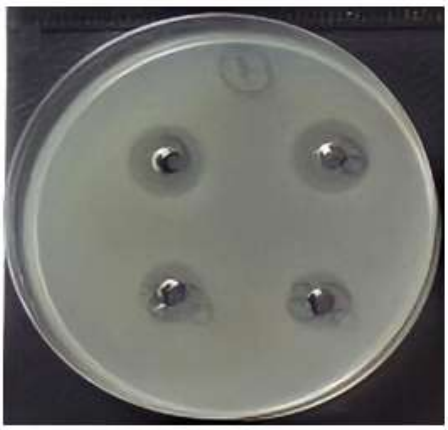
The following compounds show good activity against various bacteria
Gram Positive Bacteria
Organic solvent was showed good activity against ***staphylococcus aureus***
Aqueous solvent was showed significant activity against ***staphylococcus aureus***.

Gram negative bacteria
Organic solvent was showed good activity against ***Escherichia coli.***
Aqueous solvent was showed significant activity against ***Escherichia coli.***

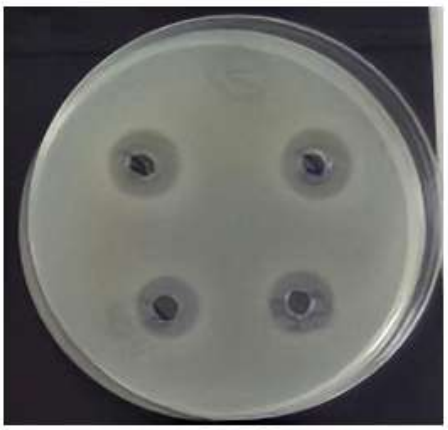
Anti-Oxidant screening

The Ethanol extract of *Andrographis Paniculata*, was screened for antioxidant activity by Free radical screening method using DPPH (*α*- diphenyl-*β*- picrylhydrazyl). All the different volumes of plant extracts were collected for absorbance of the mixture was read at 517nm. The percentage radical scavenging activity of the plant extracts was studied and it showed good activity.

ANTI-BACTERIAL SCREENING

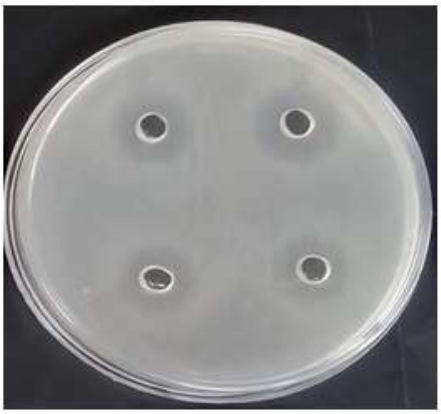


a) Staphylococcus aureus



b) E-coli (Aqueous) (Aqueous)

a) Staphylococcus aureus (Organic)



b) E-coli (Organic)

CONCLUSION

Leaf extracts from "*Andrographis Paniculata*" are becoming more and more important due to their diverse biological and pharmacological characteristics. We now report on the anti-microbial and anti-oxidant investigation of leaf extracts from "*Andrographis Paniculata"*, continuing our work on diverse extracts. The leaves were gathered, dried, and ground into a coarse powder before being extracted over a 72-hour period using a Soxhlet apparatus with an aqueous and organic solvent. Following extraction, the leftovers were gathered, dried, and used for early research on compounds like carbohydrates, terpenoids, saponins, amino acids, alkaloids, and flavonoids, among others. After that, it is employed in research on antimicrobials. The extracts were subjected to antimicrobial study. During the antimicrobial study all the different concentration solvent were found to possess moderate antibacterial and antioxidant activity.

REFERENCES

1. Bennerman R, Burton J, Chen WC, editors. Medicinal plants and primary health care: an agenda for action. Traditional medicine and health care coverage. Geneva, Switzerland: World Health Organization; 1983.
2. Mahady GB. Global harmonization of herbal health claims. J Nutr. 2001;131(3s):1120S-3S. doi: 10.1093/jn/131.3.1120S, PMID 11238830.

3. Rates SM. Plants as source of drugs. Toxicon. 2001;39(5):603-13. doi: 10.1016/s0041-0101(00)00154-9, PMID 11072038.
4. Kumar N, Wani ZA, Dhyani S. Ethnobotanical study of the plants used by the local people of Gulmarg and its allied areas, Jammu and Kashmir, India. Int J Curr Res Biosci Plant Biol. 2015;2(9):16-23.
5. Bernhoft A. A brief review on bioactive compounds in plants. In: Bioactive compounds in plants – benefits and risks for man and animals. Oslo: Norwegian Academy of Science and Letters; 2010. p. 11-7.
6. Sarker SD, Nahar L. Chemistry for pharmacy students general, organic and natural product chemistry. England: John Wiley & Sons; 2007. p. 283-359.
7. Fong HH. Integration of herbal medicine into modern medical practices: issues and prospects. Integr Cancer Ther. 2002;1(3):287-93; discussion 293. doi: 10.1177/153473540200100313, PMID 14667286.
8. Carson CF, Riley TV. Toxicity of the essential oil of Melaleuca alternifolia or tea tree oil. J Toxicol Clin Toxicol. 1995;33(2):193-4. doi: 10.3109/15563659509000474, PMID 7897762.
9. Cooper EL. Drug discovery, CAM and natural products. Evid Based Complement Alternat Med. 2004;1(3):215-7. doi: 10.1093/ecam/neh032, PMID 15841253.
10. Cooper EL. bioprospecting: the 21st century. CAM: eCAM. pyramid.Evid Based Complement Altern Med 2005;2:125–7.
11. Tsao JCI, Zeltzer LK. Complementary and alternative medicine approaches for pediatric pain: a review of the state-of-the-science. Evid Based Complement Altern Med 2005;2:149–59.
12. Solecki R. Shanidar IV, a Neanderthal flower burial in Northern Iraq.Science 1975;190:880–1.5. Bensky D, Gamble A. Chinese Herbal Medicine: Materia Medica,Revised edition. Seattle, WA: Eastland Press, Inc., 1993, 13–7.
13. Farnsworth NR, Morris RW. Higher plants—the sleeping giant of drug development. Am J Pharm Sci Support Public Health 1976;148:46– 52.
14. Bacher W. Scham als Name Palastinas. Jew Q Rev 1906;18:564–5
15. Harit Kumar R. Invitro Antimicrobial Activity of Plant Extracts by Turbidity Method (Doctoral dissertation, RVS College of Pharmacy, Coimbatore).
16. CLSI, Performance Standards for Antimicrobial Disk Susceptibility Tests, Approved Standard, 7th ed., CLSI document M02-A11. Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087, USA, 2012.
17. CLSI, Method for Antifungal Disk Diffusion Susceptibility Testing of Yeasts, Approved Guideline. CLSI document M44-A. CLSI, 940 West
18. Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898, USA, 2004
19. J. Hausdorfer, E. Sompek, F. Allerberger, et al., E-test for susceptibility testing of Mycobacterium tuberculosis, Int. J. Tuberc. Lung Dis. 2 (1998)751–755.
20. S. Magaldi, S. Mata-Essayag, C. Hartung de Capriles, et al., Well diffusion for antifungal susceptibility testing, Int. J. Infect. Dis. 8 (2004) 39–45.
21. C. Valgas, S.M. De Souza, E.F.A. Smânia, et al., Screening methods to determine antibacterial activity of natural products, Braz. J. Microbiol. 38 (2007) 369–380.
22. A.E. Jiménez-Esquilín, T.M. Roane, Antifungal activities of actinomycete strains associated with high-altitude Sagebrush Rhizosphere,
23. J. Ind. Microbiol. Biotechnol. 32 (2005) 378–381.
24. L. Elleuch, M. Shaaban, S. Smaoui, et al., Bioactive secondary metabolites from a new terrestrial Streptomyces sp. TN262, Appl. Biochem. Biotechnol. 162 (2010) 579–593.
25. Kokate, C.K., Purohit, A.P., Gokhale, S.B., In; Pharmacognosy, 39th edition, Nirali Prakashan, Pune. 2002; 106-109, 256, 461.
26. Khare RS. Globalizing South Asian Food Cultures. Curried Cultures: Globalization, Food, and South Asia. 2012 May 1; 34:237.
27. Pathak K, Das RJ. Herbal medicine-a rational approach in health care system. International Journal of Herbal Medicine. 2013; 1(3):86
28. 9.36.Mosihuzzaman M. Herbal medicine in healthcare-an overview. Natural product communications. 2012 Jun; 7(6):1934578X1200700628.
29. Calixto JB. Efficacy, safety, quality control, marketing and regulatory guidelines for herbal medicines (phytotherapeutic agents). Brazilian Journal of medical and Biological research. 2000; 33:179-89.
30. Zakaria ZA, Balan T, Suppaiah V, Ahmad S, Jamaludin F. Mechanism (s) of action involved in the gastroprotective activity of *Andrographis*

31. *Paniculata* Journal of ethno pharmacology. 2014 Feb 12; 151(3):1184-93.
32. Pereira GA, Arruda HS, de Morais DR, Eberlin MN, Pastore GM. Carbohydrates, volatile and phenolic compounds composition, and antioxidant activity of (*Andrographis paniculata* L.) fruit. Food research international. 2018 Jun 1; 108:264-73.
33. Buhian WP, Rubio RO, Valle Jr DL, Martin-Puzon JJ. Bioactive metabolite profiles and antimicrobial activity of ethanolic extracts from *Andrographis paniculata* L. leaves and stems. Asian Pacific journal of tropical biomedicine. 2016 Aug 1; 6(8):682-5.