RESEARCH ARTICLE

In-vitro studies on the primitive pharmacological activities of *Andrographis paniculata* and *Centella asiatica*

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Manuscript details:

Received: 31.01.2017
Accepted: 12.03.2017
Published : 06.04.2017

Editor: Dr. Arvind Chavhan

Cite this article as:
Nivedheedha M and Sivasubramaniyan C (2017) In-vitro studies on the primitive pharmacological activities of *Andrographis paniculata* and *Centella asiatica*, *International J. of Life Sciences*, 5 (1): 82-86.

Acknowledgement:
I am deeply grateful to UGC for providing me UGC-BSR fellowship which help me conducting the experimental work smoothly and to the Dr. OMKAR, HOD of Zoology Department, University of Lucknow for all sources and materials used for reviewed this manuscript, for his valuable support and guidance.

ABSTRACT

From ancient times, Medicinal plants were used for the treatment of various deadly diseases. Especially Asian countries like India, srilanka, Indonesia etc., were used medicinal plants as a potential curing agent for various diseases and used in their different medicinal system named ayurvedic, siddha etc.,. *Andrographis paniculata* from Acanthaceae and *Centella asiatica* from Apiaceae were make a mile stone in the traditional medicine. Both of these plants contain phytochemicals such as flavonoids, flavones, and diterpenoides etc., They have various pharmacological activities whereas in the present study we clearly investigate their antibacterial, antifungal and antioxidant activities in their leaves extracts. Our research reveals that the aqueous extract shows maximum inhibitory effect against the tested bacteria and fungi when compared to others. In addition to that we evidenced that these extracts also have antioxidant activity.

Key words: Methanol, DPPH, Chloramphenicol.

INTRODUCTION

From ancient times medicinal plants had the most important environment impact in their role to save the animals especially human beings from various deadly diseases. In that way the plant named *Andrographis paniculata* is an annual herb from the family Acanthaceae and spread all over the world especially in Asian countries such as India, Bangladesh and Indonesia (Stepanovic, 2003). They cultivated more over all parts of India and it was used to treat variety of ailments such as bronchitis, influenza, gonorrhea, diabetes, blood purifier, dysentery and diarrhea (Jada *et al*., 2007). Its leaves contain the phytochemicals such as, flavonoids; triterpenes etc., because of that it exhibits various pharmacological activities such as antioxidant, anti-inflammatory, and anti-microbial. And another environmentally important medicinal plant named *Centella asiatica (L) from the* family Apiaceae which is native to asia. The Phytochemicals triterpinoid, Asiaticoside, Asiatic acid, and madecassic acid were present in it. And its used to treat wound, mild;

duresis, alertness, antiaxety , stress, and boosting memory. In the pharmaceutical industry drug development is depend on the natural products especially the plants. Even though the origins of all modern drugs were natural product but mechanistic studies of action of natural products against the disease were very low. So our research mainly focused on to find the mechanism of the action of the drug compounds present in the *Andrographis paniculata* and *Centella asiatica* against various diseases. For that we initially plan to do the antioxidant, and anti-microbial studies of *Andrographis paniculata* and *Centella asiatica.*

MATERIAL AND METHODS

Plant collection:

The leaves used were selected from the healthy, mature and disease free *Andrographis paniculata* and *Centella asiatica* plants, Collected from Thanjavur, Tamilnadu, India. All the chemicals were analytical grade from hi-media and all the glass wares used were completely sterilized and every process was done at completely sterilized condition.

Extraction

The leaves of *Andrographis paniculata* and *Centella asiatica* were washed with distilled water and 100 gm of fresh leaves were crushed using mortar and pestle with distilled water and the extract was filtered through Whatman No. 1 filter paper and centrifuged up to the complete removal of debris. The whole process was repeated three times and finally, the concentrated extract was collected in closed container and kept in a refrigerator at 4 °C. the same process was done for the methanol extract.

Bacterial strains:

Gram Positive

Bacillus cereus, Bacillus subtilis, Staphylococcus aureus, and Staphylococcus epidermis

Gram negative

Pseudomonas aeruginosa , Salmonella typhi, E.coli,

were the bacterial strains used to check the antibacterial activity of *Andrographis paniculata* and *Centella aciatica.*

Antibacterial test:

The final solution contains 0.8 mg/50 µl concentrations, was considered as the test samples for each solvents. For the preparation of sample disc, paper discs of 5 mm diameter were made from Whattman filter paper by punch machine and then autoclaved at 121°C for 15 min. 50 µl of extract was applied to the paper discs under aseptic conditions. Blank discs were also prepared using solvents only. Chloramphenicol discs (30 µg/disc) were used as standards.

For each of the test organisms, the pre-culture was taken from stock cultures and was grown in nutrient broth at 37°C for 24 h. using sterile forceps, all the sample and blanks discs were placed on the marked positions on the seeded petri dishes maintaining an aseptic condition. The standard discs were placed separately onto another set of seeded Petri dishes. The plates were kept at 4°C for 24 h to allow sufficient time for the test material to diffuse to a considerable area of the medium. After that, they were incubated at 37°C for 24 h. The resulting clear zones were measured by a transparent scale (Arora *et al.,* 2004, Balakrishnan *et al*., 2015)

Fungal strains:

Aspergillus flavus, Colletotrichum corchori, and Fusarium equiseti were the fungal strains used to check the antifungal activity of *Andrographis paniculata* and *Centella aciatica.*

Antifungal test:

The anti-fungal screening was done by disc diffusion method. The final solution contains 0.8 mg/50 µl concentrations, was considered as the test samples for each solvents. For each of test fungi, separate plate was prepared. Similarly, another set of plates were prepared using a standard antibiotic clotrimazole at a concentration of 80 µg/ml. A set of control plates were also prepared using PDA plates alone. All of the plates were incubated at 24°C for 4 days after which the inhibition of fungal colony was measured with a transparent scale in mm and the percentage of inhibition of mycelial growth was calculated. Finally the antibiotic clotrimazole was employed as a standard for comparison (Singariya *et al*., 2014).

Antioxidant Test:

The Radical Scavenging Activity (RSA) of extracts of *Andrographis paniculata* and *Centella aciatica* was

done by using DPPH assay. Different concentrations (200, 400, 600, and 800) of these extracts mixed with methanol and adjusted to nearly 8.5 ml and .5 ml of 0.1 mM methanolic solution of DPPH was added to each tubes and vortexed. Then the tubes were kept undisturbed 20 min at room temperature. Absorbance was measured at 517nm using uv-visible spectrophotometer. RSA was expressed in inhibition percentage and calculated by a standard formula (Dhanani *et al*., 2013).

RESULTS AND DISCUSSION

Antibacterial activity:

The leaves extracts of *Andrographis paniculata* and *Centella aciatica* were tested for antibacterial activities. All the samples were tested for antibacterial activities against seven pathogenic bacteria including gram-positive and gram-negative using paper disc method and the results were discussed in Table 1 and 2. The zone less than 7mm was considered as resistant. All the studied pathogens were found to be moderately susceptible to AE and ME extracts of leaves with zone of inhibition ranging from 7 to 14 mm.

The aqueous leaf extract of *Andrographis paniculata* was found to have potential antibacterial activity against all the bacteria studied. The highest activity was found against *E.coli* (with a zone of inhibition of 17 mm respectively). Likewise the methanol extract also had the same but the highest activity was found against *Bacillus subtilis* (with a zone of inhibition 13mm).

The aqueous leaf extract of *Centella aciatica* was found to have potential antibacterial activity against all the bacteria studied. The highest activity was found against *Bacillus cereus* (with a zone of inhibition of 12mm respectively). Likewise the methanol extract also had the same but the highest activity was found against *Staphylococcus aureus* (with a zone of inhibition 13mm).

Table 1: Antibacterial activity of *Andrographis paniculata*

	Zone of inhibition		
Gram Positive			
Name of Bacteria	**Aqueous extract**	**Methanol extract**	**Chloramphenicol**
Bacillus cereus	9	7	20
Bacillus subtilis	7	13	15
Staphylococcus aureus	12	9	10
Staphylococcus epidermis	-	10	25
Gram Negative			
Pseudomonas aeruginosa	14	10	25
Salmonella typhi	8	-	20
E.coli	17	12	16

Table 2: Antibacterial activity of *Centella asiatica*

	Zone of inhibition		
Gram Positive			
Name of Bacteria	**Aqueous extract**	**Methanol extract**	**Chloramphenicol**
Bacillus cereus	12	8	20
Bacillus subtilis	9	7	15
Staphylococcus aureus	7	13	10
Staphylococcus epidermis	9	-	25
Gram Negative			
Pseudomonas aeruginosa	10	9	25
Salmonella typhi	-	7	20
E.coli	11	10	16

The above results proved that *Andrographis paniculata* and *Centella aciatica* had a stronger antibacterial activity. However, the standard antibiotic chloramphenicol showed very strong inhibition against almost all of the tested bacteria.

Antifungal activity:

The leaves of extracts of *Andrographis paniculata* and *Centella aciatica* were tested for antifungal activities. All the samples were tested for antifungal activities against three fungi using disc diffusion method and the results were discussed in Table 3 & 4. The zone less than 7mm was considered as resistant. All the studied pathogens were found to be moderately susceptible to with a zone of inhibition ranging from 7 to 14 mm.

The aqueous leaf extract of *Andrographis paniculata* was found to have potential antifungal activity against all the fungi studied. The highest activity was found against *Aspergillus flavus* (with a zone of inhibition of 13 mm respectively). Likewise, the methanol extract also had the same but the highest activity was found against *Aspergillus flavus* (with a zone of inhibition 20 mm).

The aqueous leaf extract of *Centella aciatica* was found to have potential antibacterial activity against all the bacteria studied. The highest activity was found against *Aspergillus flavus* (with a zone of inhibition of 15mm respectively). Likewise the methanol extract also had the same but the highest activity was found against *Colletotrichum corchori* (with a zone of inhibition 11mm).

The above results proved that *Andrographis paniculata* and *Centella aciatica* had a stronger antifungal activity. However, the standard antibiotic clotrimazole showed very strong inhibition against almost all of the tested fungi.

Anti-oxidant activity:

Both of the extracts and ascorbic acid showed a dose-dependent activity. The activities of all the concentration of both extracts of *Andrographis paniculata* and *Centella aciatica* was tabulated in table 4a &4b. Among them the highest scavenging activity of *Andrographis paniculata* and *Centella aciatica extracts was* 800 µg/ml concentration. The results reveals that both of the plants have strong antioxidant activity.

Table 3: Antifungal activity of *Andrographis paniculate and Centella aciatica*

Zone of Inhibition	***Andrographis paniculata***			***Centella aciatica***		
Name of fungi	**Aqueous extract**	**Methanol extract**	**Clotrimazole**	**Aqueous extract**	**Methanol extract**	**Clotrimazole**
Aspergillus flavus	13	20	35	15	9	35
Colletotrichum corchori	8	-	20	10	11	20
Fusarium equiseti	-	9	25	7	-	25

Table 4:Antioxidant activity of Ascorbic acid compared with *Andrographis paniculate and Centella aciatica*

	Andrographis paniculata		***Centella aciatica***	
Test material	**Concentration**	**Scavenging activity**	**Concentration**	**Scavenging activity**
Ascorbic acid	100	84.60	100	84.60
	200	87.70	200	87.70
	400	89.40	400	89.40
	800	92.20	800	92.20
Andrographis paniculata Aqueous extract	100	56.40	100	55.30
	200	80.40	200	72.40
	400	85.20	400	77.20
	800	86.40	800	81.40
Andrographis paniculata Methanol extract	100	54.30	100	53.40
	200	73.40	200	74.30
	400	80.20	400	82.40
	800	84.10	800	83.20

CONCLUSION

Finally we concluded that the results of the study reveal that the aqueous and methanol extracts of leaf of *Andrographis paniculata* and *Centella aciatica* exhibits very potential antimicrobial and antioxidant activity. When compared with others the aqueous leaf extract of *Andrographis paniculata* have strong antimicrobial and antioxidant activity. These results can be the strong scientific evidence for the use of this plant as a useful source of antioxidant, antibacterial and antifungal references. Furthermore, because of their extensive pharmacological activity the two plants are considered as environmentally important one. In order to find the other activities of these plants we extended our research to next level that is in- vivo studies.

Conflicts of interest: The authors stated that no conflicts of interest.

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