

Investigation on Phytochemical and Antimicrobial Properties of *Andrographis Paniculata*

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Abstract

Marine microbes signify a capable source of beneficial products expecting unearthing for the inhibition or handling of numerous pathogens. In this research article the bio active complex between marine isolate and well known herbal *Andrographis paniculata* was synthesized by active fraction that showed maximum activity on total antioxidant and DPPH in dose dependent manner. The bio active complex ratio (5:5) showed antibacterial potency at low dose against *Salmonella typhi* and *E. coli* with zone of inhibition 21mm and 20mm respectively. The synergistic activity can be achieved by the complexation between marine and herbal sources.

Keywords: *Andrographis Paniculata*, antimicrobial, herbal, phytochemical

Introduction

The curiosity in medicinal herbs has grown significantly over the past twenty years, through the usage of herbal-based supplements as organic and for self-medication via both the wider populace, to the research journal of the physiological activities of plants on humans. There is a broad trend to use herbal medicines to complement the diet outside these medicinal approaches to plants, primarily with the goal of enhancing the quality of care and combating elderly person's diseases. In conventional and complementary medicines, India was already described as a major resilient region worldwide.

Andrographis paniculata belongs to the family of Burm and Wall, for instance, Nees originated from the family of Acanthaceae, which is considered as a King of Bitters, in Tamil it is known as Nilavempu, is a yearly herbaceous flora that is cultivated widely in South Asia, China and areas of Europe. With respect to conventional medicine, *A. Paniculata* is commonly utilized to avoid common cold, upper respiratory tract diseases like sinusitis and illness (Gabrielian, et. al., 2002) and antidote to toxins from snakes and insecticides (Gabrielian, et. al., 2002) (Samy, et. al., 2008). In vivo and in vitro viz., antimicrobial (Singha, et. al., 2003; Mishra, et. al., 2009), anti-viral (Wiert, et. al., 2005), anti-inflammatory (Wen, et. al., 2010), anti-HIV (Human Immunodeficiency Virus) (Chao, et. al., 2010), immunomodulatory/immune-stimulatory (Calabrese, et. al., 2000) and anti-cancer (Calabrese, et. al., 2000) have been demonstrated to display different modes of biological activity (Iruetagoiena, et. al., 2005; Li, et. al., 2007). The flora demonstrated possible treatment intervention in the management of liver disorders, common cold, and human coughs (Geethangili, et. al., 2008). The existence in *A.* of major

bioactive compounds. Paniculata make plants valuable for the treatment of various ailments and also have the capacity to provide productive human drugs. To set standards for crude products, the quantitative estimation of pharmacokinetic specifications would aid (Sharma, et. al., 2012). An antioxidant are compounds effective of preventing the chain responses that destroy cells through eliminating free-radical intercedes, and prevent another oxidation responses by relieving anxiety accountable for many neurodegenerative conditions. *Andrographis paniculate* Nees, a multi-purpose tropical flora is supposed to have antioxidant characteristics (Mishra, et. al., 2013). Its significance in the pharmaceutical drug arena has been greatly increased by the distinctive 2° metabolites present throughout this plant. In this present investigation, individual potency of various bioactive and therapeutic metabolites of herbal source can be get active complex have been achieved against clinical infections.

MATERIALS AND METHODS

COLLECTION OF PLANTS

Collection of plant source

A complete plant of *A. paniculata* was reclaimed from the National Institute of Siddha (NIS), Chennai and authentication was done by CAS in Botany, University of Madras, Tamil Nadu.

New and healthy leaf, stem, and roots were utilized to extract biologically active portions of *A. paniculata*. The fragments of floras that were utilized for the solvent was cleaned using H₂O to eliminate soil and dirt substances. Further, it was dry underneath shaded place. Dry substances were mixed to form a finer powdery and stored in air tightening bottles (BabyShalini, 2015).

Preparation of Organic solvent extracts

To prepare organic solvent methanolic extract, 25 g powdery of *Andrographis paniculata* leaves was placed in each solvent for repeated 3 days at room T and sieved. The filtrate was centrifuge at 3000 rpm for 15 mins and disappeared to aridness in the water bath. The extract was stored at 4°C in air (Daniyan, 2008; Chattopadhyay *et al.*, 2009).

Phytochemicals Screening

Phytochemicals are secondary metabolites that synthesized from primary metabolites of the plant. Phytochemicals consist of several chemical classes such as terpenoids, phenols, flavonoids, saponins and tannins that have several biological effects on microorganisms (Yadav and Agarwala, 2011).

Total antioxidants

The total antioxidant of *A. paniculata* extract was estimated by ABTS (2, 2'-azino-bis 3ethylbenzenethiazoline-6-sulphonic acid) method. The reaction mixture was prepared by adding

25ml of plant extract, 1 ml of diluted ABTS solution and 5ml of distilled H₂O and the mix was permitted to kept for 5 mints. The absorbency of the mixture was calculated at 734nm utilizing spectrophotometry towards reagent blank. A total antioxidant was estimated by comparing the absorbance with standard chart prepared using b-carotene.

Thin Layer Chromatography (TLC)

The methanolic extract obtained from *A. paniculata* was dissolved in respectively solvent. Each test samples were applied through a fine capillary tube onto a pre-covered silica gel 60 F254 TLC plate. The plates were established with the mobile phase in a twin-trough compartment by using different mobile phase system for different extract. The TLC plate was observed below shorter wavelength (254 nm) and longer wavelength (365 nm) of UV-light and was photographed. The R_f value was determined by using the following formula (Gujjeti and Mamidala, 2013).

Evaluation of antimicrobial activity

The Antimicrobial activities of methanolic extract were examined by agar well diffusion method (FirdausJahan., 2011). Pathogenic microbial species followed using *Escherichia coli* MTCC 64, *Salmonella typhi* MTCC 734, *Klebsiella pneumoniae* MTCC 10 and *Staphylococcus aureus* MTCC 96 were utilized. The bacteria were injected in nutrient medium and stand instantaneous at 37°C for exponential development of cultured medium. In forthcoming years, the microbes were washed of purely processed Mueller-Hinton agar dishes and well generated of 6 mm in the dish. The plant extract was loaded different concentration of 1mg/mL. Then the plates were nurtured at 37°C for 24h and further the zone of inhibition was calculated by employing the Antibiotic zone scale (Himedia, India) and +ve control used as ciprofloxacin.

Results and discussions

The floral extracts were separated for the occurrence of major 2° metabolite groups like Alkaloides, Flavonoides, Saponin, Terpenoide, Tannin, Glycosides, Phytosterol, and Proteins, as per general phyto-chemical techniques. The experiments were depending on visual remark of the changing in color or development of precipitate later the introduction of precise reagent. The results of phytochemical tests carried out for *Andrographis paniculata* with different solvents and the present study exhibited the presence and absence of phytochemical compounds in each solvent extract. It was found that Methanol showed maximum number of phytochemicals when compared to Chloroform, Petroleum ether and Acetone (Table.1).

Table 1: Preliminary Phytochemicals Screening of *Andrographis paniculata* extract

Phytochemicals	Ethyl acetate extract
Flavanoids	Present
Phenols	Present
Tannin	Absent

Saponin	Absent
Sugars	Present
Terpenoids	Present
Glycosides	Present
Steroids	Present

Thin Layer Chromatography (TLC)

The TLC images of the *A. paniculata* extract viewed under short and long UV wavelength are shown in (Fig.1). A total number of four spots have been observed on chloroform, and methanol ratio. Ethyl acetate extract of *A. paniculata* yielded the maximum four spots on TLC because, the incidence of countless 2° metabolites like phenol, flavonoids and glycosides etc. responsible for the color producing behavior against ultraviolet radiation table 3. The retention factor, Rf values of all solvent extracts was shown in Table 2.

Distance travelled by Solvent front = 5.3cm

Distance travelled by Spot 1 = 4.4cm

Distance travelled by spot 2 = 3.6cm

Distance travelled by spot 3 = 3.2 cm

Distance travelled by spot 4 = 1.4cm

Table 4: TLC identification of *A. paniculata*

Spot	Rf values
Spot 1	0.83
Spot 2	0.67
Spot 3	0.60
Spot 4	0.26

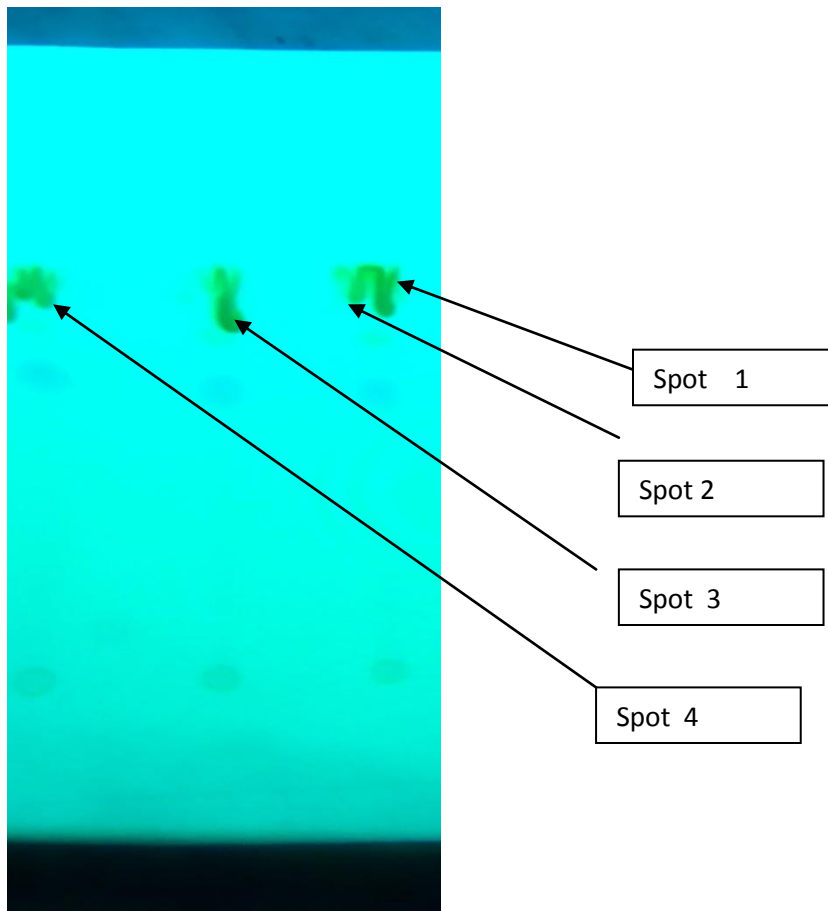


Fig 1: Diverse fractions from *A. paniculata* ethyl acetate extract

Table 5: Total antioxidant activity of fractions *A. paniculata*

Fraction	Total Anti Oxidant Activity				
	100µg/ml	200µg/ml	300µg/ml	400µg/ml	500µg/ml
AF-1	45.553±0.01	63.597±0.01	68.331±0.46	75.152±0.58	79.087 ±0.86
AF-2	72.889±0.12	78.067±0.05	83.301±0.07	89.241±0.19	90.438±0.37
AF-3	34.879.06	42.098±0.71	58.123±0.05	65.126±0.88	70.089 ±0.09
AF-4	20.893±0.08	34.729±0.09	50.391±0.08	57.202±0.51	64.238±0.97
Quercetin	75.09±0.01	82.621±0.03	84.203±0.57	87.02 ± 0.01	92.08 ± 0.45

Value represents mean ± SD

Table 6: Inhibition of DPPH Activity of the fractions of *A. paniculata*

Fractions	Percentage Inhibition of DPPH Activity				
	10µg/ml	20 µg/ml	30µg/ml	40 µg/ml	50µg/ml
AF1	30.53±0.067	48.73±0.051	54.38±0.101	58.92±0.504	62.56±0.091
AF2	50.34±0.150	55.71±0.18	67.98±0.79	78.81±0.41	88.76±0.16
AF3	20.13±0.027	38.43±0.055	44.36±0.201	55.42±0.804	58.56±0.045
AF4	34.23±0.097	37.65±0.871	44.98±0.109	50.43±0.565	56.69±0.901
Vitamin C	60.92±0.01	72.69±0.02	84.75±0.01	94.53±0.01	98.57±0.01

Value represents mean ± SD

The fractions of herbal *Andrographis paniculata* showed the potential response against the free radicals. The fraction AF2 (Table: 5, 6) of *Andrographis paniculate* ethyl acetate extract exhibited maximum activity on both total antioxidant activity and DPPH inhibition in dose dependent manner and thus the reason the fraction was selected for antimicrobial action towards human selected pathogens. The trial organisms like *Escherichia coli* MTCC 64, *Salmonella typhi*

MTCC 734, *Klebsiella pneumoniae* MTCC 10 and *Staphylococcus aureus* MTCC 96 were used and the highest inhibition on *E. coli* (20mm), and *Salmonella typhi*(21mm) respectively. The herbal *Andrographis paniculata* contained much secondary metabolites like andrographolide (Mishra *et al.*, 2007) and it produced notable activity against clinical pathogens this was higher than the inhibitory activity of *Andrographis paniculate* solvent extracts against the clinical pathogens Fig.2 (Baby Shalini, 2015).

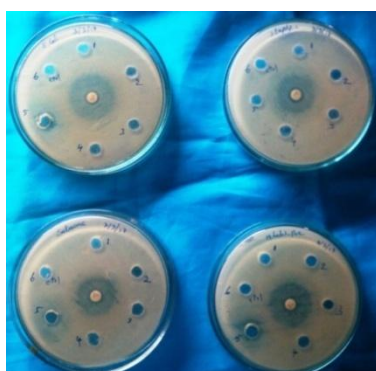


Fig 2: Antibacterial activity of fraction AF2

Table 7: Antibacterial activity of fraction AF2

ratio	Zone of inhibition (mm)			
	<i>Escherichia coli</i> MTCC 64	<i>Staphylococcus aureus</i> MTCC 96	<i>Salmonella typhi</i> MTCC 734	<i>Klebsiella pneumoniae</i> MTCC 10
1:9	10	8	8	12
9:1	11	11	10	8
3:7	10	13	10	11
7:3	12	14	11	10
5:5	20	10	21	19
Ciprofloxacin	23	23	22	23

Conclusion

It can be inferred from the comprehensive literature review and clinical outcomes examination, which *Andrographis paniculata* is a conventional treatment for fever, cough, and numerous illnesses. In tumors, immune modulatory function, and viral infections and others, it also takes various immune functions. The flora is also useful for the treatment of cardiovascular disorder and the prevention of liver damage, thus enhancing heart and liver functions. In our experiment the plant extract was scrutinized for the occurrence of major 2° metabolite groups like Flavonoids, Phenols, Tannin, Saponin, Sugars, Terpenoids, Glycosides and Steroids as per

common phyto-chemical techniques. The experiments were based on visual opinion of the changing in color or creation of precipitate once the introduction of particular reagent. The present study exhibited the presence and absence of phytochemical compounds in each solvent extract. It was found that Methanol showed maximum number of phytochemicals when compared to Chloroform, Petroleum ether and Acetone. The fraction study made an attention on different combinations of herbal fractions which exhibited the high potential activity on evaluating parameter such as free radical scavenging and antibacterial activity. It could be supported as a secure, highly essential medicinal properties for humanity, going to take care of the promising functions of the plant.

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