PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITY OF *ANDROGRAPHIS PANICULATA, CENTELLA ASIATICA* AND *WITHANIA SOMNIFERA* LEAVES

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Abstract: Although, Indian traditional medicinal system is emerging one in which formulations and specific drugs were studied for the specific targets in disease and disorders. In order to the support this we have taken three medicinal plants namely *Andrographis paniculata, Centella asiatica* and *Withania somnifera*. Qualitative phytochemical screening and antimicrobial activities were studied for the aqueous and methanolic leaf extracts of these plants. The results revealed the positive outcomes of the extracts as a suitable drug candidate for pathogenic infections caused by *Bacillus subtilis, Pseudomonas aeruginosa, Candida albicans*, and *Aspergillus niger*. In future, it may go further formulations for the better effect against human pathogens.

Index terms: **Chemical constituents, antibacterial, antifungal, *andrographis paniculata, centella asiatica, withania somnifera.***

1. INTRODUCTION

Ayurveda is one of the traditional medicinal methods for curing various diseases since more than 5000 years (Balakrishnan P, *et al.,* 2015). Medicinal plants are an important episode in the medical sector. Around 5 000 species have specific therapeutic value among 250 000 higher plant species on earth (Roy DC *et al.,* 2013). *Eclipta alba, Clerodendrum phlomidis, Ocimum basilicum, Ficus racemosa, Nigella sativa, Leucas aspera, and Adhathoda vasica* were the most important medicinal value plants in which current researches are focused on (Balakrishnan p *et al.,* 2018; Mohanmarugaraja MK *et al.,* 2017; Balakrishnan P *et al.,* 2018). In the pharmaceutical industry drug development is mainly depends on the natural products especially the plants. Even though the origins of all modern drugs were the natural product but mechanistic studies of the action of natural products against the disease were very low (Atanasov AG *et al*., 2015). So our research mainly focused on to find the mechanism of the action of the drug compounds present in the *Andrographis paniculata*, *Centella asiatica* and *Withania somnifera* against various diseases.

Andrographis paniculata which belongs to the Acanthaceae family is an annual herb spread all over the world especially in Asian countries such as India, Bangladesh, and Indonesia (Stepanovic, 2003). It is used to treat a variety of ailments such as cancer, diabetes bronchitis, gonorrhea, dysentery and diarrhea (Jada *et al.,* 2007). It also possesses some pharmacological activities such as antiproliferative, antimicrobial, antioxidant, anti-inflammatory and anti-ulcer. These activities are due to the presence of phytochemicals such as alkaloids, flavonoids, and triterpenes present in it.

Centella asiatica which belongs to the family Apiaceae family is a perennial herb which is native to Southeast Asia. In Ayurveda, it is used to treat leprosy, skin diseases, asthma, bronchitis, elephantiasis, eczemas, ulcers, anxiety, urethritis, cataract, eye troubles, diarrhea, and wound healing (sharan V *et al.,* 2011). It contains phytochemicals such as alkaloids, glycosides, terpenoids, steroids, flavonoids and tannins which are the most active plant molecules to work against cancer (Arumugam T *et al.,* 2011).

Withania somnifera (Ashwagandha) an annual herb Solanaceae family has widely spread all over the world especially in Asia (Joshi P, *et al*., 2014). It has been also used in Ayurvedic, Siddha and Unani as pharmaceuticals and nutraceuticals. It possesses some pharmacological activities such as anti-inflammatory, anti-cancer, cardioprotective, anti-stress, anti-diabetic, anti-oxidant, immunomodulatory, neuroprotective, anti-microbial, and rejuvenating properties of WS (Dar *et al.,* 2015). It is also used to treat nervous, digestive, circulatory, respiratory, reproductive and urinary disorders (G. Singh *et al.,* 2010). Preliminary studies have found various constituents of ashwagandha exhibit a variety of therapeutic effects with little or no associated toxicity.

Nowadays, Anti-microbial compounds are of key players in treating various ailments because of their increased resistance to microorganisms which are pathogenic in nature and harmful to humans (Saranya *et al.,* 2017; Sundaramoorthy M *et al.,* 2014). For this reason, there is a need for the development of compounds with fewer side effects and more targeted action on the microorganisms. Still, research is going on to identify compounds with anti-microbial activity mainly from plant origin as they have lesser side effects (Mamedov N., 2012). Most of the researchers concentrate on screening and minimum inhibitory

concentration (MIC) determination of extracts of plants rather than identifying compounds with activity, which makes most of the researches unuseful for drug discovery (Ramena G *et al.*, 2018).

2. MATERIALS AND METHODS

2.1. Chemicals and glassware

The chemicals used for the entire study was purchased from Hi-media (analytical grade) and the glassware was completely sterilized.

2.2. Plant collection

The matured healthy leaves of *Andrographis paniculata, Centella asiatica and Withania somnifera (L.),* were collected and also checked for disease-free plants from a local garden in Thanjavur, Tamilnadu, India.

2.3. Extraction

Accurately 100 grams of *Andrographis paniculata, Centella asiatica (L.)* and *Withania somnifera* leaves were shade dried and weighed. Aqueous and methanol were used as solvents (because most of the plant-based molecules were polar in nature) in which the soaked samples allowed to homogenize (i.e., grinding in mortar and pestle) and kept undisturbed for 24 h. Then the extract was filtered using Whatman No. 1 filter paper and the filtrate was stored at 4°C (Nagarasan S *et al.,* 2016).

2.4. Qualitative Determination of Chemical Constituents

Table.1: Qualitative Determination Procedures (Ramalingam PS *et al.*, 2017)

S.NO	CHEMICAL CONSTITUENTS	REAGENT MIXTURE	AMOUNT OF EXTRACTS (ml)	CONFIRMATION
1	Terpenoids	2 ml of chloroform + conc.H_2SO_4 (after the addition of extracts)	2	The appearance of reddish brown color.
2	Flavonoids	3 drops of 10% lead acetate	1	The appearance of yellow color.
3	Saponins	5 ml of distilled water + 3 drops of olive oil (shook vigorously)	5	Formation of the oil emulsion.
4	Tannins	2 ml distilled water + 3 drops of 0.1% ferric chloride (after the addition of extracts)	2	The appearance of green color.
5	Alkaloids	3 drops of Hager's reagent	2	Formation of a yellow precipitate
6	Steroids	2 ml of chloroform + 5 drops of conc.H_2SO_4	2	A reddish brown ring was formed
7	Glycosides	2 ml of chloroform + 2 ml of acetic acid	2	A color change from Violet to blue to green.
8	Phlobatannins	2 ml of1% HCl	2	Formation of the red precipitate.
9	Proteins	1 ml of conc.H_2SO_4	2	Formation of a white precipitate.
10	Coumarins	3 ml of 10% NaOH	2	The appearance of yellow color.

2.5. Antimicrobial activity

In present days, pathogens are getting lethal and more virulent by the change in environmental conditional. In order to the susceptibility of a drug, it is most important to check their activity against human pathogens (Sethuraman J *et al.,* 2017). So, we concentrated on testing these extracts in some bacteria and fungi to check its efficacy.

2.5.1. Antibacterial

The aqueous, and methanolic leaf extracts of *Andrographis paniculata, Centella asiatica,* and *Withania somnifera* were prepared at a concentration of 0.8 mg/50 µl. The anti-bacterial screening was done by disc diffusion method by using Chloramphenicol as standard. Paper discs of 5 mm dia were made from Whattman No.1 filter paper and autoclaved at 121°C for 20 min. At aseptic conditions, 50 µl of extracts were applied to the paper discs and standard (30µg/disc) was also prepared. For the antibacterial test, *Bacillus subtilis* (Gram-positive) and *Pseudomonas aeroginosa (*Gram-negative) strains were cultured and plated in nutrient agar medium. All the samples and standards along with blank were placed on the marked positions on the Petri dishes maintaining an aseptic condition. Then 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 using a transparent scale (Meignanalakshmi *et al*., 2013; Samy and Ignacimuthu 2000).

2.5.2. Antifungal

The aqueous, and methanolic leaf extracts of *Andrographis paniculata, Centella asiatica,* and *Withania somnifera* were prepared at a concentration of 0.8 mg/50 µl. The anti-fungal screening was done by disc diffusion method by using clotrimazole as standard. Paper discs of 5 mm dia were made from Whattman No.1 filter paper and autoclaved at 121°C for 20 min. At aseptic conditions, 50 µl of extracts were applied to the paper discs and standard (30µg/disc) was also prepared. For the antifungal test, *Candida albicans* and *Aspergillus niger* strains were cultured and plated in PDA medium. All the samples and standards along with blank were placed on the marked positions on the Petri dishes maintaining an aseptic condition. 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. After that, they were incubated at 37°C for 24 h. (P Singariya *et al.,* 2014; Nagarasan S *et al.,* 2016).

3. RESULTS AND DISCUSSION

3.1. Qualitative Determination of Phytochemical Constituents

Table 2: Chemical Constituents Presence in Plant Extracts

S.NO	Chemical constituents	*Andrographis paniculata* Leaf extracts		*Centella asiatica* Leaf extracts		*Withania somnifera* Leaf extracts	
		Aqueous Extract	Methanol Extract	Aqueous Extract	Methanol Extract	Aqueous Extract	Methanol Extract
1	Alkaloids	+	+	-	+	+	+
2	Flavonoids	+	+	+	+	+	+
3	Carbohydrates	+	+	+	+	+	-
4	Protein	+	+	+	+	+	+
5	Phenols	+	+	+	-	+	+
6	Saponins	+	-	+	+	+	+
7	Tannins	+	+	-	+	+	+
8	Phytosterols	+	-	+	-	+	+
9	Terpenoids	+	+	+	+	+	-
10	Phlobatannins	+	-	-	-	-	-

The above table represents the Qualitative determination of phytochemical constituents present in the aqueous and methanolic extracts of *Andrographis paniculata, Centella asiatica* and *Withania somnifera* leaves. It represents that, the presence of all type of chemical constituents were present in the aqueous extracts of *Andrographis paniculata* followed by *Withania somnifera* and *Centella asiatica*. In spite in methanolic extracts, mostly alkaloids, flavonoids, carbohydrates, proteins and tannins were present and the others were in different compositions with respect to the concentration of the extracts.

3.2. Antimicrobial Activity

3.2.1. Antibacterial Activity

Table.3: Antibacterial Zone of Inhibition Values of Plant Extracts

Sample type	Plant extract/ standard	Zone of Inhibition (mm)	
		***Bacillus subtilis* (Gram +ve)**	***Pseudomonas aeruginosa* (Gram -ve)**
Andrographis paniculata leaves	Aqueous Extract	8	10
	Methanolic Extract	14	16
	Chloramphenicol	50	38
Centella asiatica leaves	Aqueous Extract	16	12
	Methanolic Extract	13	12
	Chloramphenicol	56	36
Withania somnifera leaves	Aqueous Extract	-	8
	Methanolic Extract	11	9
	Chloramphenicol	16	26

The Aqueous and methanolic extracts of *Andrographis paniculata*, *Centella asiatica* and *Withania somnifera* leaves were tested against *Bacillus subtilis and Pseudomonas aeruginosa* for antibacterial activity. Generally, Zone of inhibition of 7 mm is considered to be an active one. The results revealed that all the extracts except aqueous extracts of *Andrographis paniculata* and *Withania somnifera* have a potent activity against *Bacillus subtilis*. Aqueous extract of *Centella asiatica* and methanolic extract of *Andrographis paniculata* has more activity. In Pseudomonas aeruginosa, methanolic extract of *Andrographis paniculata* showed a strong inhibition. However, the standard antibiotic chloramphenicol showed very strong inhibition against both bacteria.

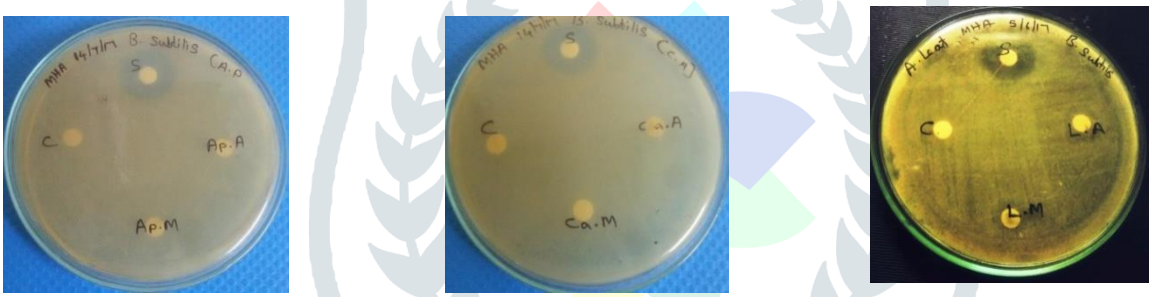

Fig.1: Antibacterial activity of *Andrographis paniculata*, *Centella asiatica*, *Withania somnifera* leaf extracts against *Bacillus subtilis.*

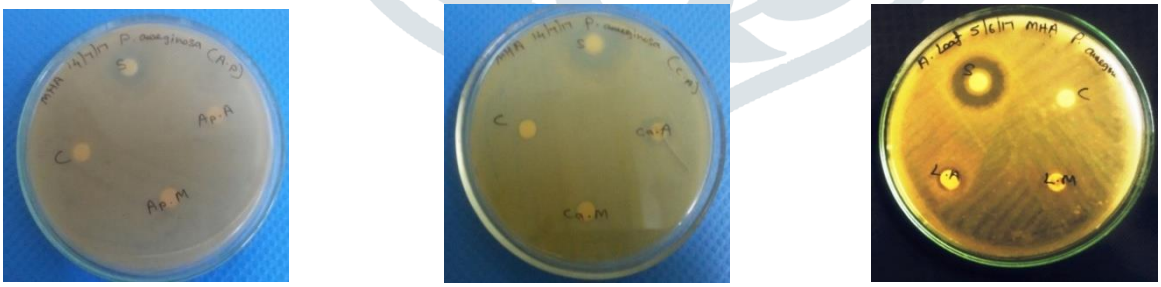

Fig.2: Antibacterial activity of *Andrographis paniculata*, *Centella asiatica*, *Withania somnifera* leaf extracts against *Pseudomonas aeruginosa.*

3.2.2. Antifungal Activity

Table.4: Antibacterial Zone of Inhibition Values of Plant Extracts

Sample type	Plant extract/ standard	Zone of Inhibition (mm)	
		Candida albicans	***Aspergillus niger***
Andrographis paniculata leaves	Aqueous Extract	11	10
	Methanolic Extract	5	6
	Clotrimazole	60	65
Centella asiatica leaves	Aqueous Extract	12	10

	Methanolic Extract	6	7
	Clotrimazole	62	68
Withania somnifera leaves	Aqueous Extract	10	9
	Methanolic Extract	17	11
	Clotrimazole	40	13

The Aqueous and methanolic extracts of *Andrographis paniculata, Centella asiatica* and *Withania somnifera* leaves were tested against *Candida albicans and Aspergillus niger* for antibacterial activity. Generally, Zone of inhibition of 7 mm is considered to be an active one. The results revealed that all the extracts except methanolic extracts of *Andrographis paniculata* and *Centella asiatica* have a potent activity against *Candida albicans.* Aqueous leaf extract of *Andrographis paniculata* has more activity against *Candida albicans.* While, both the Aqueous leaf extract of *Andrographis paniculata* and *Centella asiatica* showed a greater activity against *Aspergillus niger*. Moreover, all the studied pathogens were found to be moderately susceptible to all aqueous leaf extracts with a zone of inhibition ranging from 7 to 14 mm. However, the standard antibiotic Clotrimazole showed very strong inhibition against almost all of the tested fungi.

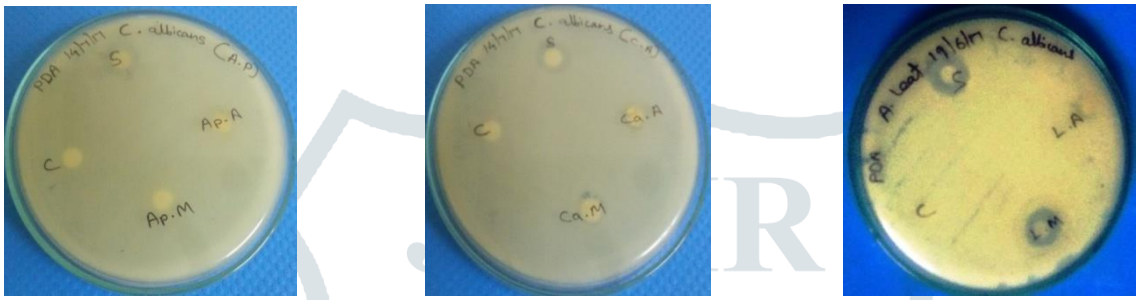

Fig.3: Antifungal activity of *Andrographis paniculata, Centella asiatica, Withania somnifera* leaf extracts against *Candida albicans.*

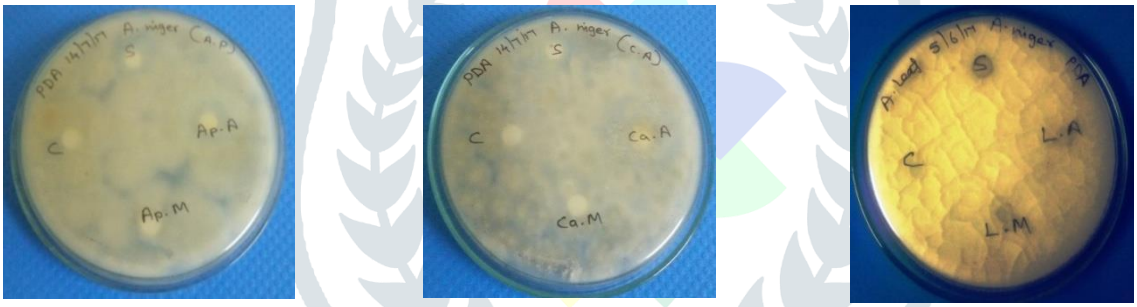

Fig.4: Antifungal activity of *Andrographis paniculata, Centella asiatica, Withania somnifera* leaf extracts against *Aspergillus niger.*

4. CONCLUSION

Historically, medicinal plants were the richest source of successful drugs and formulations and identification of new pharmacological products. This study reveals the occurrence of chemical constituents and the antimicrobial activities of the aqueous and methanolic extracts of *Andrographis paniculata, Centella asiatica,* and *Withania somnifera* leaves. The extracts had more potent activity against those virulent pathogens and this may lead to the separation of the specific bioactive compound from the extracts. While natural product-based drug discovery will be an important source for the development of new drugs in the future.

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