



Study of antioxidant and antimicrobial activities of *Andrographis paniculata*

***Suparna Deepak, Asmita Pawar and Punam Shinde**

*Department of Biotechnology, Karmaveer Bhaurao Patil College, Vashi, Navi Mumbai,
Maharashtra, India*

ABSTRACT

*An antioxidant is a molecule capable of terminating the chain reactions that damage cells by removing free radical intermediates, and inhibit other oxidation reactions by thereby reducing stress responsible for many degenerative disorders. *Andrographis paniculata* Nees, a multipurpose tropical plant is believed to have many medicinal properties. The purpose of this study was to evaluate the in vitro antioxidant activity of the Hexane, DCM and Methanol extracts of *Andrographis paniculata* Nees. The antioxidant activity of the extracts was evaluated using two assays viz. DPPH Radical Scavenging Assay and Total Reducing Capacity. In DPPH assay, the IC₅₀ values obtained for Hexane, DCM and Methanol extracts were 223.3 µg/ml, 69.32 µg/ml and 82.23 µg/ml respectively. In the Total reducing capacity assay, activity increased in dose dependent manner for all the three plant extracts. The antimicrobial activity of the extracts was evaluated using the Broth Dilution Method. The results obtained in the present study indicate that the leaves of *Andrographis paniculata* showed the best antibacterial activity against the gram positive organisms ie; *S.aureus* and *S. pyogenes*. The DCM extract showed the lowest MIC (100 µg/ml) for both the gram positive organisms. All the 3 extracts showed antifungal activity against *C. albicans* with 200 µg/ml as the MIC.*

Keywords: kalmegh, phytoconstituents, HPTLC, DPPH and antibacterial.

INTRODUCTION

In Ayurvedic Medicine, there are numerous herbs which have been used historically for treating a large variety of ailments. Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs.

Andrographis paniculata Nees. is a plant that has been effectively used in traditional Asian medicines for centuries. The plant belongs to the family of Acanthaceae. The common name is Kalmegh or bhumimin. It is an annual, branched, erect- running ½ to 1 meter in height. Kalmegh is a plant of Indian Origin which is used since decade in Aurvedic Medicine purpose. Mainly it is available in Indian and Sri Lanka. Wild variety of the plant is available in West Bengal, Orissa, Bihar, Jharkhand, Andhra Pradesh, Assam, Kerala, Karnataka, and Utter Pradesh. It can be cultivated in whole India but the plain are more suitable for high production and Commercial cultivation. Kalmegh is an annual plant with three feet height. The leaves are 7.5 Cm Long and 2.5 Cm wide. The flowers of plant is of white colour .The seeds are small in size of yellowish brown in colour. The test is of plant is extra bitter that's why it is called as "King of Bitters" [1]. *A. paniculata* has been reported as having antibacterial, antifungal, antiviral, choleric, hypoglycemic, hypocholesterolemic, and adaptogenic effects [2].

Oxidative stress and antioxidants

Free radicals are chemical species possessing an unpaired electron that can be considered as fragments of molecules and which are generally very reactive. Reactive free radicals formed within cells can oxidize bio-molecules and lead to cell death and tissue injury [3]. Overproduction of Reactive free radicals results in oxidative stress that can be an important mediator of damage to cell structures, including lipids and membranes, proteins, and DNA. An antioxidant is a molecule capable of inhibiting the oxidation of other molecules. They terminate the chain reactions that damage cells by removing free radical intermediates, and inhibit other oxidation reactions. They do this by being oxidized themselves, so antioxidants are often reducing agents such as thiols, ascorbic acid or polyphenols [4]. Antioxidants help in preventing degenerative diseases such as cancer, coronary heart disease and even altitude sickness [5].

The purpose of the study is to study the phytochemical and antimicrobial properties of *Andrographis paniculata* Nees. leaves and obtain the HPTLC fingerprints of the same followed by the evaluation of in-vitro antioxidant activity of the Hexane, DCM and Methanol extracts of *Andrographis paniculata*.

MATERIAL AND METHODS

i) Sample Collection and Extraction:

The leaves of the plant *Andrographis paniculata* were collected from Sunrise Agriland Development And Research Pvt. Limited, Jaipur. The leaves were washed and shade dried for 2 weeks. Then they were powdered using a grinder, sieved and used for further analysis. The plant material was subjected to Soxhlet extraction using three different solvents successively in order of polarity i.e. from non-polar to polar. The solvents used were hexane, dichloromethane and methanol.

ii) Phytochemical Analysis:

Preliminary phytochemical screening was studied by the method described earlier for all the three crude extracts of *A. paniculata* for detecting the presence of Alkaloids, Tannins, Flavonoids, Steroids, Terpenoids, Saponins, Phenols, Antraquinones, Phlobatannins and Organic Acids [6, 7].

iii) Fingerprint Analysis by HPTLC:

The analysis was just a preliminary analysis since standard markers were not used to confirm the presence of active phytoconstituents. 3 crude extracts (Hexane, DCM and Methanol) were dissolved in their respective solvents (50µg in 5ml). Pre-coated silica gel plates of 200mm thickness were used for thin layer chromatography (TLC). Ethyl acetate: Formic acid: Glacial acetic acid: Water (10: 0.5: 0.5: 1) was used as mobile solvent. Anisaldehyde sulphuric acid was used as the Derivatizing agent.

iv) Screening for the Antioxidant Activity

I. DPPH Radical Scavenging Assay

The 3 plant extracts were dissolved in their respective solvents (5mg/ml) and 7 concentrations (10 to 70 µg/ml) were made using methanol. In 200 µl of this volume, 1800 µl of DPPH (0.0002%) was added and all the tubes were incubated in dark at room temperature for 30 mins. O.D. was taken at 517 nm. The standard used was Butylated Hydroxyl Toluene (5mg/ml) using methanol as diluent. The percent (%) inhibition by sample treatment is determined by comparison with the control using the following formula:

$$\% \text{ inhibition of DPPH activity} = (A - B) / A * 100$$

Where, A = optical density of the blank

B = optical density of the sample

IC₅₀ values denote the concentration of sample which is required to scavenge 50% DPPH free radicals.

II. Total Reducing Capacity

The 3 plant extracts were dissolved in their respective solvents (5mg/ml) and 6 concentrations (10 to 60 µg/ml) were made using Phosphate buffered saline (PBS). In this solution, 2.5 ml of potassium ferri cyanide (1%) was added and the tubes were incubated in the water bath at 50°C for 20 mins. After cooling it, 2.5 ml of trichloroacetic acid (10%) was added and then centrifuged at 3000 rpm for 10 mins. 2.5 ml of supernatant was pipetted out in tubes and were

mixed with 2.5 ml of distilled water and 1 ml of freshly prepared Ferric chloride solution (0.1%). Then all the tubes were incubated for 20-30 mins and O.D. was taken at 700 nm. The standard used was Ascorbic acid (5mg/ml) using methanol as diluent.

v) Antimicrobial Activity Screening:

I. Antibacterial Assay

In vitro antibacterial activity was examined for hexane, DCM and methanol extracts of *A. paniculata* by using the Broth Dilution method [8]. The 3 extracts were added in Mueller Hinton Broth in respective large test tubes with 3 different concentrations (100, 200 & 500 µg/ml) of hexane, DCM and methanol extracts of *A. paniculata*. These tubes were inoculated with 0.1 ml of two Gram-positive bacteria were *Staphylococcus aureus* ATCC25923, *Streptococcus pyogenes* ATCC21059, while two Gram-negative bacteria were *Escherichia coli* ATCC25922 and *Pseudomonas aeruginosa* ATCC27853. All the tubes were incubated at 37°C for 24 hours and the microbial growth was monitored turbidometrically [9].

II. Antifungal Assay

In vitro antifungal activity was examined for hexane, DCM and methanol extracts of *A. paniculata* by using the Broth Dilution method. The 3 extracts were added in Potato dextrose broth in respective large test tubes with 3 different concentrations (100, 200 & 500 µg/ml) of the hexane, DCM and methanol extracts of *A. paniculata*. The tubes were inoculated with 0.1 ml of two fungal strains - *Candida albicans* ATCC7596 and *Aspergillus niger* ATCC9763. All the tubes were incubated at room temperature for 48 hours.

RESULTS AND DISCUSSION

I. Phytochemical Analysis

Table 1: Observation table of Phytochemical Analysis

Sr. No.	Tests	Hexane Extract	DCM Extract	Methanol Extract
1.	Alkaloids	-	+	+
2.	Tannins	+	+	-
3.	Flavonoids	+	+	+
4.	Steroids	+	+	+
5.	Terpenoids	-	-	-
6.	Saponins	-	-	-
7.	Phenols	+	+	+
8.	Antraquinones	-	+	-

The phytochemical screening on quantitative analysis shows that the leaves and stems of *A. paniculata* are rich in flavonoids, alkaloids, steroids, phenols and tannins. These phytochemicals confer antimicrobial activity on the plant extract.

II. HPTLC Analysis

Table 2: The HPTLC fingerprint analysis of various extracts of *A. paniculata* Nees

Sr. No.	Extract	Detection Wavelength (nm)	No. of spots	R _f values
1.	Hexane	366	4	0.47, 0.84, 0.87 and 0.93
2.	Dichloromethane	366	9	0.05, 0.18, 0.30, 0.48, 0.58, 0.69, 0.78, 0.86 and 0.89
3.	Methanol	366	4	0.06, 0.19, 0.84 and 0.89

1)Hexane Extract

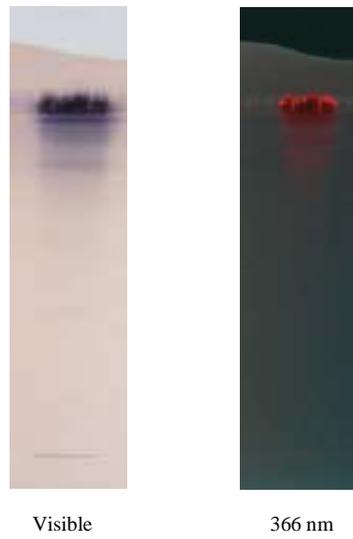
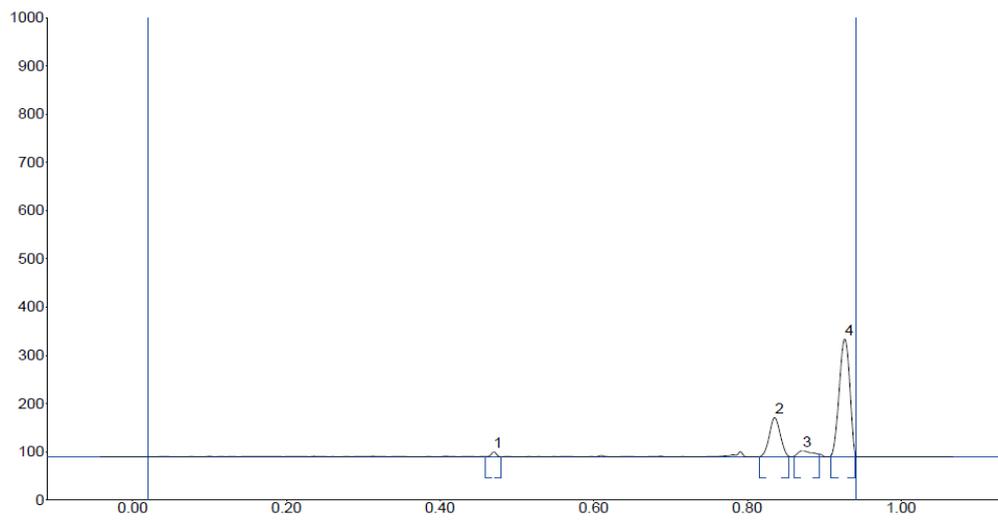


Fig. No. 1: Chromatogram of Hexane extract for flavonoids



Graph No. 1: Graph of Chromatogram of Hexane extract seen under 366 nm

2)DCM Extract

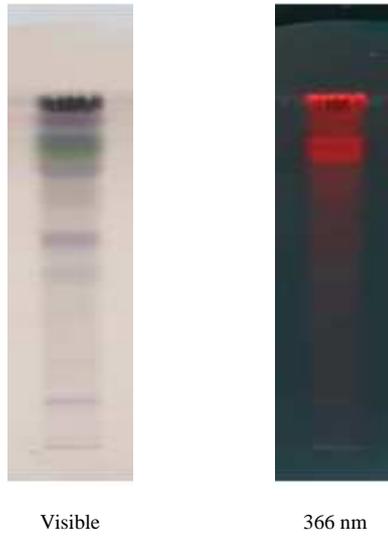
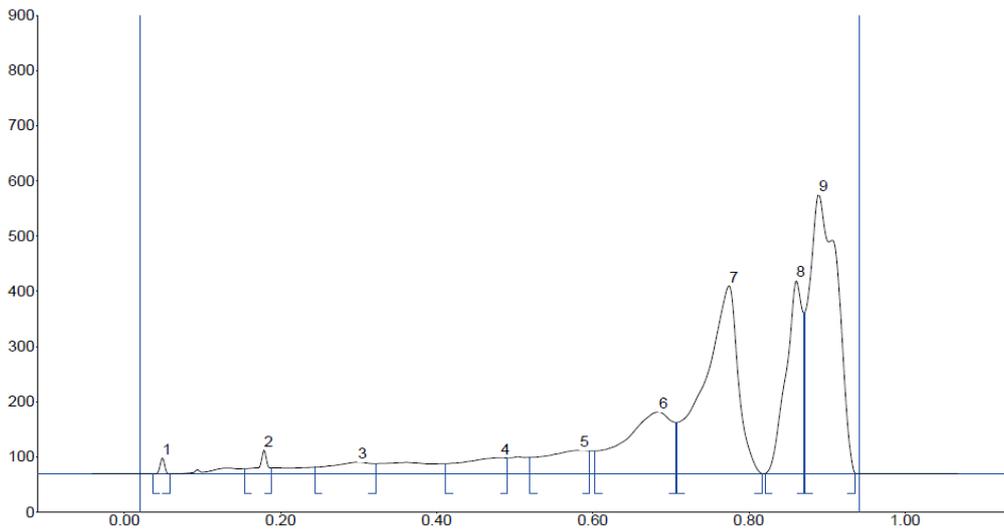


Fig No. 2: Chromatogram of DCM extract for flavonoids

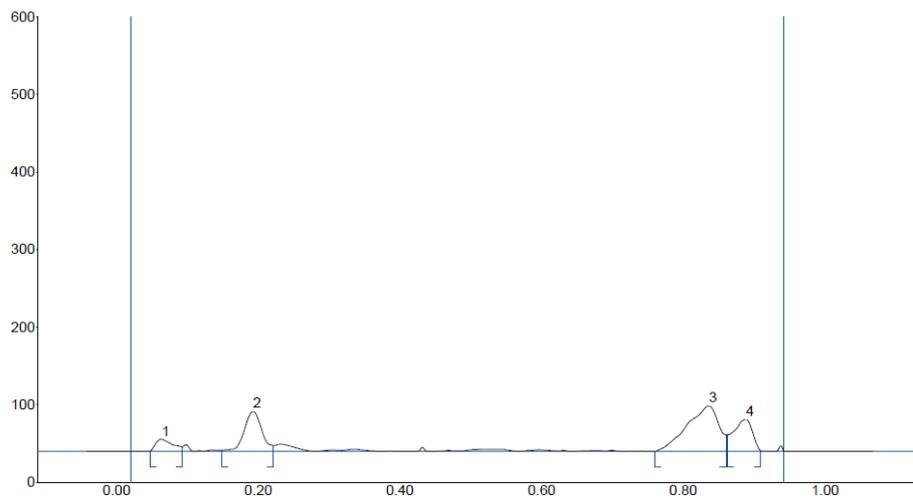


Graph No. 2: Graph of Chromatogram of DCM extract seen under 366 nm

3)Methanol Extract



Fig No. 3: Chromatogram of Methanol extract for flavonoids

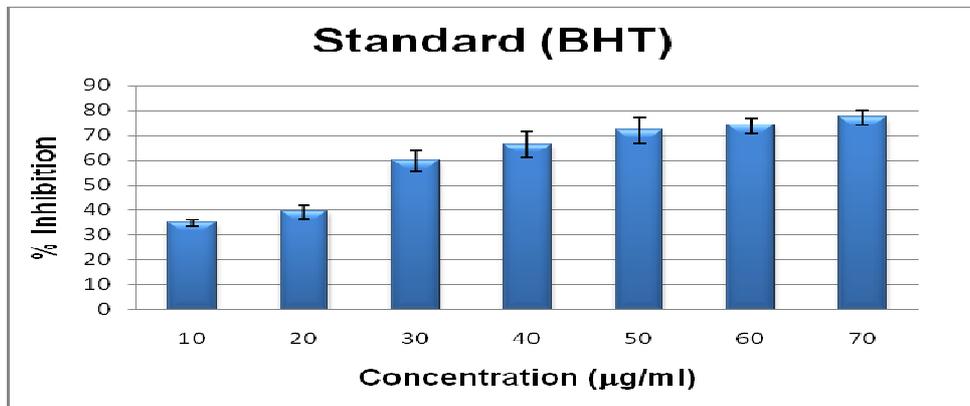


Graph No. 3: Graph of Chromatogram of Methanol extract seen under 366 nm

III. Antioxidant Assay

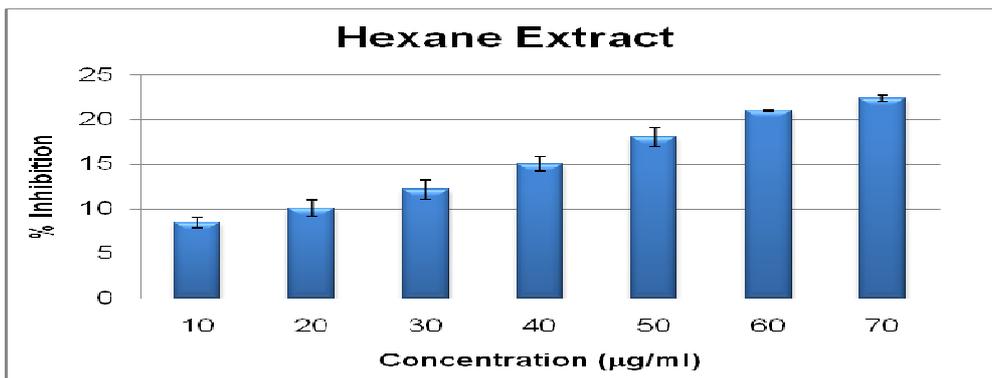
i) DPPH Radical Scavenging Assay : Scavenging capacity of ascorbic acid was measured using a spectrophotometer at 517 nm.

1) Standard (BHT)



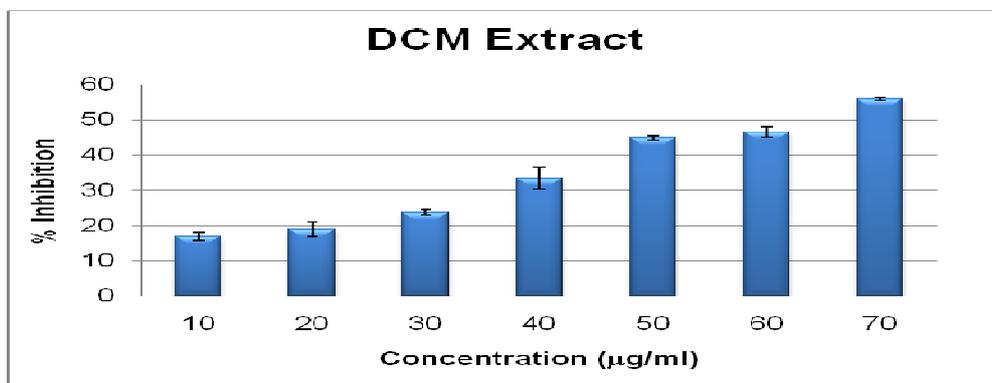
Graph No. 4: DPPH assay of BHT

2) Sample (Hexane extract)



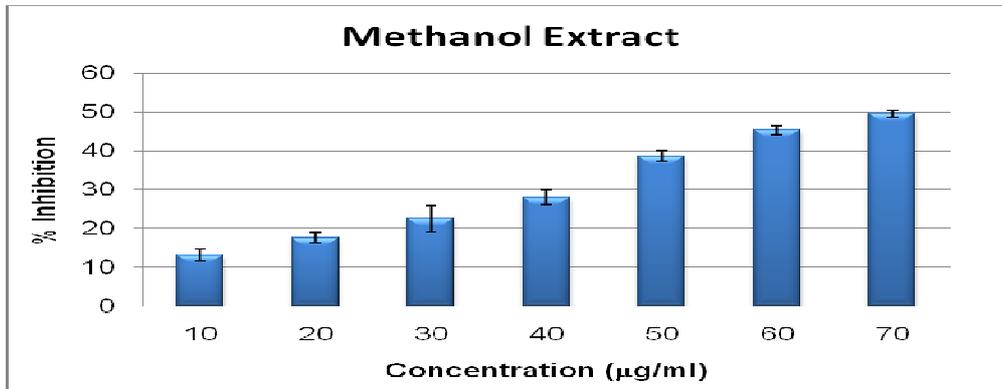
Graph No. 5: DPPH assay of Hexane extract

3) Sample (DCM extract)



Graph No. 6: DPPH assay of DCM extract

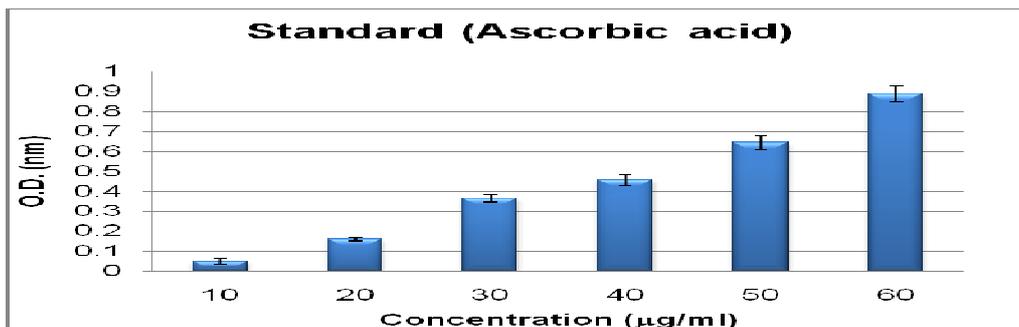
4)Sample (Methanol extract)



Graph No. 7: DPPH assay of Methanol extract

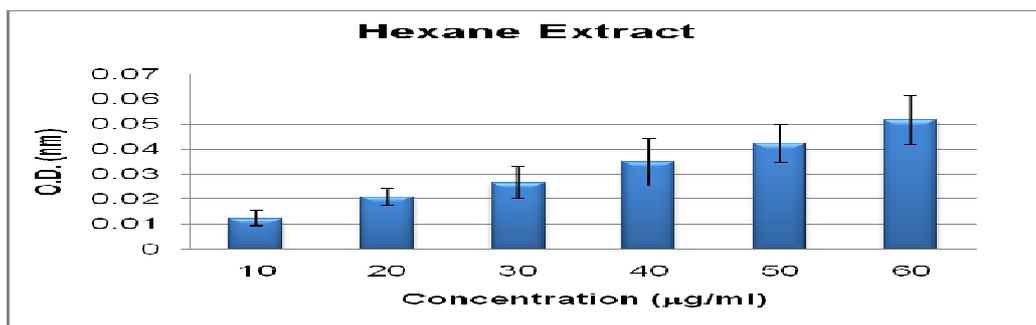
ii) Total Reducing Capacity: Total reducing capacity was measured using a spectrophotometer at 700 nm.

Standard (Ascorbic acid)



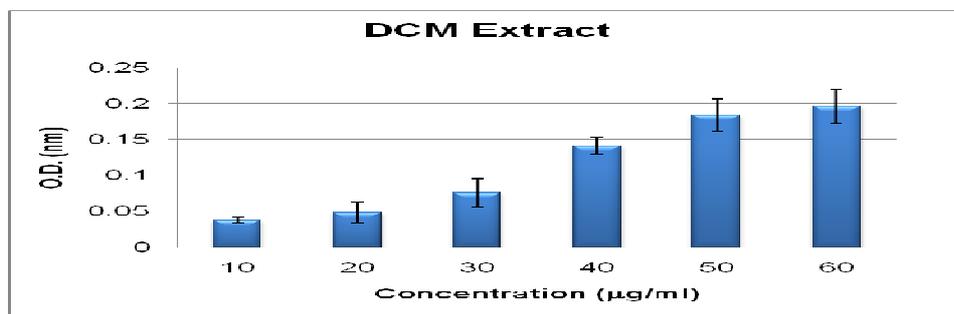
Graph No. 8: Total Reducing Capacity of Ascorbic Acid

1)Sample (Hexane extract)



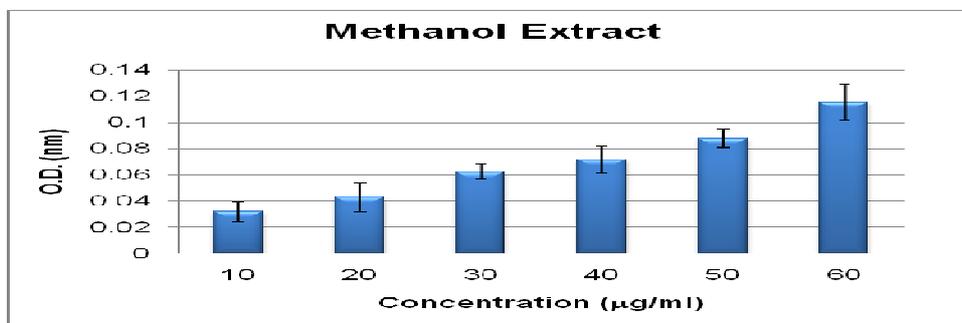
Graph No. 9: Total Reducing Capacity of Hexane extract

2) Sample (DCM extract)



Graph No. 10: Total Reducing Capacity of DCM extract

3) Sample (Methanol extract)



Graph No. 11: Total Reducing Capacity of Methanol extract

IV. Antibacterial and Antifungal Activity

Table 3: The antibacterial and antifungal activity of various extracts of *A. paniculata* Nees.

Sr. No.	Culture	Concentration of extract	Result		
			Hexane Extract	DCM Extract	Methanol Extract
1.	<i>E. coli</i>	100 µg/ml	-	-	-
		200 µg/ml	-	+	-
		500 µg/ml	+	+	-
2.	<i>P.aeruginosa</i>	100 µg/ml	-	-	-
		200 µg/ml	-	-	-
		500 µg/ml	-	-	-
3.	<i>S. aureus</i>	100 µg/ml	-	+	-
		200 µg/ml	+	+	+
		500 µg/ml	+	+	+
4.	<i>S.pyogenes</i>	100 µg/ml	-	+	-
		200 µg/ml	-	+	+
		500 µg/ml	+	+	+
5.	<i>C. albicans</i>	100 µg/ml	-	-	-
		200 µg/ml	+	+	+
		500 µg/ml	+	+	+
6.	<i>A. niger</i>	100 µg/ml	-	-	-
		200 µg/ml	-	-	-
		500 µg/ml	-	-	-

DISCUSSION

India is a varietal emporium of medicinal plants and is one of the richest countries in the world in regard to genetic resources of medicinal plants. India has about 45,000 plant species and among them many have been claimed to possess medicinal properties [10]. The need for scientific validation of these useful medicinal plants is very essential [11]. Many of these medicinal plants possess a number of properties such as anti-diabetic, antioxidant, anticancer and anti-inflammatory etc. Although, modern synthetic drugs are mostly used, the use of herbal drugs is well accepted and a continuous high demand for plant material and extracted natural products can be observed.

The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial agents has led to the screening of several medicinal plants for their potential antimicrobial activity [12].

In Ayurveda, the plant *Andrographis paniculata* Nees. has importance in maintaining human health. Qualitative analysis carried out for the 3 extracts viz. Hexane, DCM and Methanol extracts of the leaves of *Andrographis paniculata* showed the presence of phytochemical constituents. Preliminary phytochemical screening of the plant leaves revealed the presence of flavonoids, alkaloids, steroids, phenols and tannins.

Qualitative chromatographic analysis of these extracts using thin layer chromatography was performed to separate and identify the single or mixture of constituents in each extract. HPTLC fingerprint is one of the versatile tool for qualitative and quantitative analysis of active constituents [13]. It is also a diagnostic method to find out the adulterants and to check the purity.

There are numerous reports supporting the use of flavonoids as antioxidants or free radical scavengers [14]. The presence of flavonoids in the leaves of *Andrographis paniculata* enables them to be used as an antioxidant. The alkaloids are nitrogenous compounds that function in the defence of plants against herbivores and pathogens, and are widely exploited as pharmaceuticals, stimulants, narcotics, and poisons due to their potent biological activities [15]. The most important function of phenolics is in the plant defence against pathogens and thus they are applied in the control of human pathogenic infections [16]. Tannins act as antiseptic agents because of the presence of the phenol group. In ayurveda various tannin rich formulations are used for the treatment of many infections [14]. The presence of alkaloids, phenolics and tannins may explain the antibacterial and antifungal activity exhibited by the leaves of *Andrographis paniculata*.

The leaves of *Andrographis paniculata* showed the best antibacterial activity against the gram positive organisms ie; *S.aureus* and *S. pyogenes*. The DCM extract showed the lowest MIC (100µg/ml) for both the gram positive organisms. Low degree of inhibition was observed against *E.coli* whereas no effect was seen on *P.aeruginosa*. All the 3 extracts showed antifungal activity against *C.albicans* with 200 µg/ml as the MIC. No effect was observed against *A.niger*. Hence, *Andrographis paniculata* could be explored for a wide spectrum antibiotic.

Further studies on the isolation and identification of specific phytoconstituents can be undertaken which may help in understanding their role in many pharmacological actions.

CONCLUSION

The results showed the presence of many phytochemicals amongst which flavonoids were the predominant which could be responsible for the antioxidant property of the same. The phytochemical analysis of the extracts of *Andrographis paniculata* leaves. The extracts of *Andrographis paniculata* leaves showed considerable anti-oxidant activity by DPPH scavenging assay and Total reducing capacity. The best results were obtained with DCM extract showing the lowest IC₅₀ value of 69.32 µg/ml. The IC₅₀ value can be further reduced by using purified extracts. The total reducing capacity increased in dose dependent manner for all the three extracts. The DCM extracts of leaves of *A. paniculata* shows a better antibacterial activity against the gram positive organisms.

The present study of antioxidant and in-vitro antimicrobial evaluation of *Andrographis paniculata* forms a primary platform for further phytochemical and pharmacological studies.

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