Asian J. Pharm. Res. Vol 6, Issue 1, 7-11, 2016.

e-ISSN 2231 – 363X
Print ISSN 2231 – 3621

Asian Journal
of
PHARMACEUTICAL RESEARCH
Journal homepage: - www.ajprjournal.com

IN VITRO ANTIBACTERIAL AND PRELIMINARY PHYTOCHEMICAL STUDIES OF *ANDROGRAPHIS PANICULATA* (BURM. F.) NEES

Ignace Kindo* S. John Britto, R.R.Marandi, Mariat George and Emasushan Minj

The Rapinat Herbarium and Centre for Molecular Systematics, St. Joseph's College (Autonomous) Tiruchirappalli, Tamilnadu, India.

ABSTRACT

Medicinal plants are in use for long to cure ailments in the tribal area. *Andrographis paniculata* is selected for the antibacterial and phytochemical analysis on the basis of medicinal folklore reports and literature data. *A. paniculata* of Acanthaceae is an annual herb which is widely cultivated in Southern Asia, India, China and some parts of Europe. The extracts of Acetone, aqueous, chloroform, ethanol, methanol and petroleum ether solvents were subjected to qualitative phytochemical screening and antibacterial activity using standard procedures. Results showedthe presence of phytoconstituents such as Alkaloids, Glycoside, carbohydrate, flavonoids, phenols, saponins, sterols, terpenoids and tannins. Among thirteen bacterial strains tested, eight were found to be positive while the others were negative. The highest activity was recorded in methanol extract followed by ethanol, chloroform, acetone and aqueous (Table 2, Chart 1, Plate 1). The diversity of phytochemicals present suggests that *A.paniculata* could serve as a source of useful drugs.

Key words: Antibacterial, Phytochemical, Medicinal Plant, *Andrographis paniculata* (Burm. fil.) Nees.

INTRODUCTION

Medicinal plant based drugs have the added advantage of being simple, effective besides offering a broad spectrum of activity with greater emphasis on preventive action [1]. In the last century approximately 121 pharmaceutical products were formulated based on the traditional knowledge obtained from various sources [2]. Several phytochemical studies have been carried out in different parts of the world [3, 4, 5] therefore, characterization of extracts of medicinal plants is necessary, due to its numerous benefits to science and society.

A.paniculata has been used for centuries as a medicinal herb for the treatment of upper gastrointestinal tract and upper respiratory infections, fever, herpes and other chronic diseases. It has a broad range of pharmacological effects. The primary medicinal component of *A. paniculata* is andrographolide, which is a diterpene lactone. Andrographolide has been noted for its anti-cancer [6], Cardioprotective [7] and hepatoprotective properties [8]. Hence the present study was carried out to explore the phytoconstituents and antimicrobial activity of *A. paniculata* using various phytochemical techniques as well as disc diffusion method for antimicrobial studies.

MATERIAL AND METHODS

Collection and Authentication

The plant was collected from Balrampur District, Chhattisgarh, India. The taxonomic identification of the plant was carried out by Dr.S.John Britto, Director and Head, The Rapinat Herbarium and Centre for Molecular systematics St. Joseph's College (*Autonomous*) Tiruchirappalli, India. The voucher specimen was deposited at the centre (RHT 65698).

Extraction Procedure

The whole plant was dried at room temperature, then powdered, which was then stored in air tight container till use. The powdered material was weighed in a selected quantity and was subjected to Rotary shaker extraction using solvents as Acetone, Aqueous, Chloroform, Ethanol,

Corresponding Author :- **Ignace Kindo** Email:- kindoignace37@gmail.com

Methanol and Petroleum ether respectively. The solvent was then evaporated to get dry powder. The dried powder was preserved in an airtight bottle. The crude extracts thus obtained were used for further investigation of phytochemical and antibacterial studies.

Test's for Alkaloids

Hager's Test: To 2 ml extract, was addedfew drops of Hager's reagent (Saturated solution of picric acid). Formation of yellow colour precipitate signified positive result.

Mayer's Test: To 2ml extract, was added few drops of Mayer's reagent. Formation of cream precipitate indicated the presence of alkaloids.

Wagers Test: To 2ml extract, were added afew drops of Wagers reagent. Formation of reddish brown precipitate indicated the presence of alkaloids [9].

Test's for Carbohydrate

Benedict's test: Extract was treated with Benedict's reagent and heated gently. Orange red precipitate indicated the presence of reducing sugars.

Fehling's Test (For reducing sugars): Extract was hydrolysed with dil. HCL, neutralized with alkali and heated with Fehling's A&B solutions. Formation of red precipitate indicated the presence of reducing sugars.

Molisch's Test: 2ml extract + 10ml H2O + 2 drops Ethanolic α-naphthol (20%) + 2ml conc.H2SO4. Reddish violet ring at the junction.

Test's for Flavonoids

Alkaline Test: To 2-3 ml of extract, few drops of 5% NaOH solution were added. Formation of intense yellow colour which turned colourless on addition of few drops of dilute HCL indicated the presence of flavonoids [10].

Lead acetate Test: 1ml extract was treated with 1ml 10% lead acetate (pb(OAC)4) solution. Formation of yellow colour precipitate indicated the presence of flavonoids.

Pews Test: To 2-3ml extract, was added zinc powder in a test tube, followed by drop wise addition of conc. HCL. Formation of purple red or cherry colour indicated the presence of flavonoids [11].

Shinoda Test: To 2-3ml extract, few fragments of magnesium metal were added in a test tube, followed by drop wise addition of conc. HCL. Formation of red or crimson red colour indicated the presence of flavonoids [10].

Test's for Glycosides

Glycosides Test: To small amount of extract,was added 1ml water and shaken well. Then aqueous solution of NaOH was added. Yellow colour appeared that indicated the presence of glycosides [12].

Keller-Kiliani Test: (Test for cardiac glycoside): To 2ml extract, was added 1ml glacial acetic acid, one drop 5% FeCl3 and 1ml conc.H2SO4. A brown ring of the interface indicated the presence of deoxysugar characteristics of cardenolides, cardiac glycosides [10, 11].

Molisch's Test: To 1ml of extract, 2drops of Molisch's reagent was added in a test tube and 2ml of concentrate H2SO4 was added carefully keeping the test tube slightly curved. Formation of violet ring at the junction indicated the presence of glycosides [10].

Test's for Phenol

FeCl3: To 2ml of extract was added 2-3 drops of 5% ferric chloride solution. Formation of bluish-black colour showed presence of phenol and black colour showed tannins.

K2Cr2O7:To the extract was added 5% potassium dichromate solution. Positive result was confirmed by a formation of brown precipitate.

Test's for Saponins

Foam Test: 2ml extract was diluted with 10ml of distilled water and warmed gently. It was shaken for 15 minutes. Persistent froth indicated the presence of saponins [10].

NaHCO3 Test: To extract a drop of sodium bicarbonate was added. The mixture was shaken vigorously and kept for 3 min. A honey comb like froth was formed and it showed the presence of saponins.

Test's for Starch

Iodine Test:2ml extract was treated with 5 drops of Iodine solution, and blue colour indicated the presence of starch.

Test's for Steroids

Salkowski's Test: To 2ml of extract, was added 2ml chloroform and 2ml conc.H2SO4 from the side of the test tube. Chloroform layer appeared red and acid layer showed greenish yellow fluorescence indicated the presence of sterols [10].

Test's for Tannins

Braymer's Test: 2ml extract + 2ml H2O + 2-3 drops FeCl3 (5%). Green precipitate showed the presence of tannins.

FeCl3 Test: To 2ml of extract was added 2-3 drops of 5% ferric chloride solution. Formation of black colour showed the presence of tannins.

Lead acetate Test: Few drops of 10% lead acetate solution were added into 5ml of extract. Formation of yellow or red precipitate indicated the presence of tannins [12].

Test's for Terpenoids

Salkowski's Test:2 ml of chloroform and 1ml of conc.H2SO4 was added to 1ml of extract and observed for reddish brown colour that indicated the presence of terpenoids.

Asian J. Pharm. Res. Vol 6, Issue 1, 7-11, 2016.

Test Micro-organisms

13 Bacterial strains were used in study namely *Staphylococcus aureus* (MTCC # 3163), Escherichia coli (MTCC# 199), *Klebsiella pneumoniae* (MTCC # 3040), *Pseudomonas aeruginosa* (MTCC # 2474), *Salmonella paratyphi* (MTCC # 734), *Vibrio cholerae* (ATCC # 14104), *Enterobacter aerogenes* (MTCC # 2990), *Streptococcus pneumonia*e (ATCC # 7066), *Bacillus subtilis* (MTCC # 441), B. cereus (ATCC # 4342), Proteus vulgaris (MTCC # 1771), P. mirabilis (MTCC # 1429) and *Serratia marcescens* (MTCC # 2645). These pathogenic micro-organisms were obtained from Rapinat Herbarium and centre for molecular systematic, St. Joseph's College Tiruchirappalli, Tamilnadu. All the test bacterial strains were maintained on nutrient agar media at 4 °C.

Preparation of Disc

6 mm discs were prepared and sterilized in autoclave and were soaked in different extracts like Acetone, Distilled water, chloroform, Ethanol, Methanol and Petroleum ether. The standard drug streptomycin was used as control.

Determination of Antibacterial Activity

Antibacterial activities of the *A. paniculata* extract were determined by disc diffusion method [13]. Nutrient agar was prepared for the study. Each plate of Nutrient agar was swabbed with each bacterial strain by using sterile cotton swab. The soaked dried discs were placed on the surface of each inoculated plate. The plates were allowed for diffusion for half an hour and then transferred to incubator at 370c for 24 hours. Standard disc of Streptomycin was also placed as positive control. The antibacterial activity of *A. paniculata* whole plant extract was determined by measuring the diameter of zone of inhibition in mm.

RESULT AND DISCUSSION

The results obtained for qualitative screening of phytochemicals in whole plant of *A.paniculata* is presented in Table 1. Of the ten phytochemicals screened, nine were found present they are Alkaloids, Glycoside, carbohydrate, flavonoids, phenols, saponins, sterols, terpenoids and tannins. On the other hand results obtained from antibacterial screening showed the highest antibacterial activity on methanolic extract followed by ethanol, chloroform, acetone and aqueous extracts, Table 2, Chart 1, and Plate 1.

Table 1. Phytochemical screening of *A.paniculata* Whole plant

S.No.	Phytoconstituents	Name of Tests	Solvents used					
			Acetone	Aqueous	Chloroform	Ethanol	Methanol	Petroleum ether
1.	**Alkaloids**	Hagers	+	++	-	++	+++	-
		Mayers	-	+	-	-	++	-
		Wagers	-	+++	-	+	++	-
2.	**Carbohydrate**	Benedict's	-	+	-	-	-	-
		Fehling's	-	+	-	-	-	-
		Molisch's	++	++	-	+++	+++	-
3.	**Flavonoids**	Alkaline	+	++	-	++	+++	-
		Lead acetate	-	++	-	++	+++	-
		Pews	-	-	-	-	-	-
		Shinoda	-	-	+++	-	-	-
4.	**Glycosides**	Keller-Kiliani	+	+	+++	++	++	+
		Glycosides	-	++	-	++	++	-
		Molisch's	-	-	-	+++	++	-
5.	**Phenol**	FeCl3	++	++	+	++	+++	-
		K2Cr2O7	++	+++	-	+	++	-
6.	**Saponins**	Foam	-	++	-	-	-	++
		NaHCO3	++	+	-	+++	++	-
7.	**Starch**	Iodine	-	-	-	-	-	-
8.	**Steroids**	Salkowski's	++	+	+++	+	++	++
9.	**Tannins**	Braymer's	+	++	++	+	+++	-
		FeCl3	++	++	+	++	+++	-
		Lead acetate	-	++	-	++	+++	-
10.	**Terpenoids**	Salkowski's	++	+	+++	+	++	++

- Absent, + Present, ++ good, +++ very good.

Asian J. Pharm. Res. Vol 6, Issue 1, 7-11, 2016.

Table 2. Antibacterial assays of *A.paniculata* Whole plant

S.No.	Test microorganisms	Inhibition zones (mm)				
		Acetone	Aqueous	Chloroform	Ethanol	Methanol
1.	*Bacillus cereus*	0.0	0.0	0.0	9.5±0.70	12.33±1.53
2.	*Enterobacteraerogenes*	8.33±1.53	7.33±0.58	10.33±1.53	7.67±0.58	10.67±1.52
3.	*Escherichia coli*	0.0	0.0	0.0	8.33±1.52	11.33±1.52
4.	*Klebsiella pneumoniae*	18±1	0.0	17±1	11±1.52	10.67±1.53
5.	*Proteus vulgaris*	14.67±0.58	8.33±1.53	0.0	16.33±1.33	15.67±0.58
6.	*Salmonella paratyphi*	0.0	0.0	8.33±1.53	7.67±0.58	7.33±0.58
7.	*Staphylococcus aureus*	7.33±0.58	0.0	0.0	18.33±0.58	15.33±1.58
8.	*Streptococcus pneumoniae*	7.33±0.58	0.0	7.67±0.58	7.67±0.58	8±1.73

Fig 1. Antibacterial Activity of *A.paniculata* Whole plant

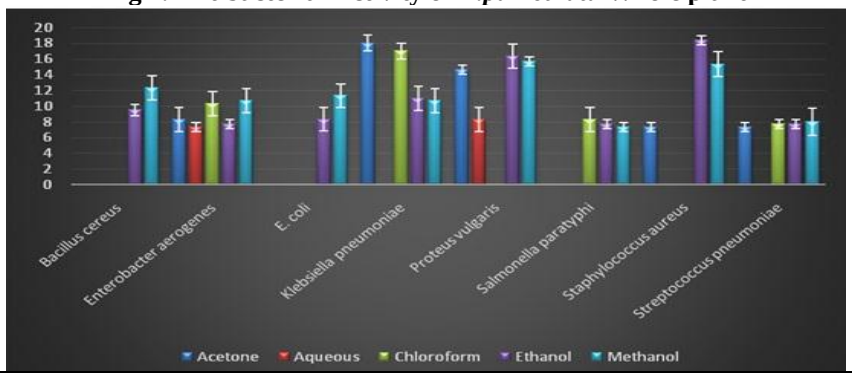
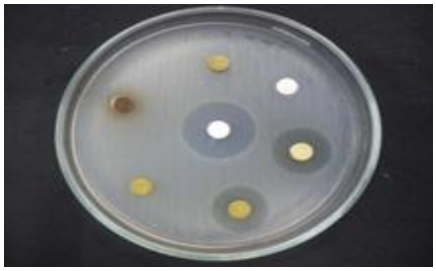


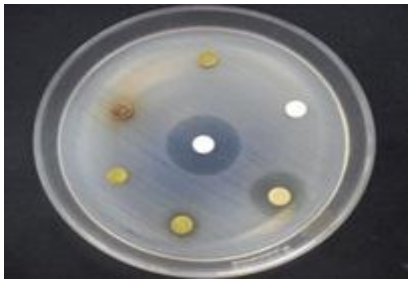
Fig 2. Antibacterial activity of *A.paniculata* Whole plant

Bacillus cereus

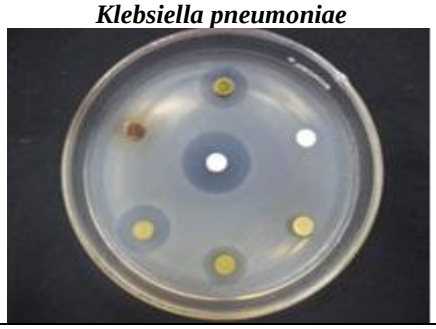


Enterobacter aerogenes

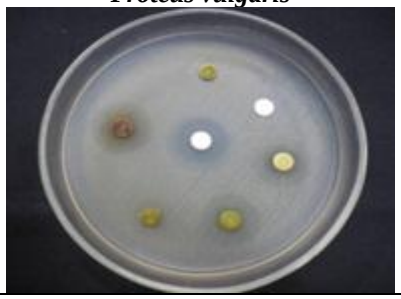
Escherichia coli



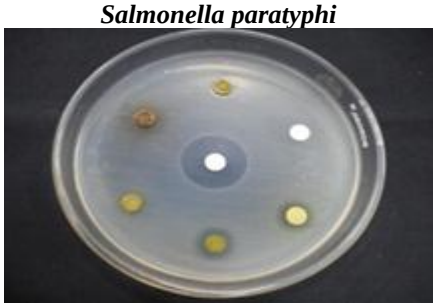
Klebsiella pneumoniae



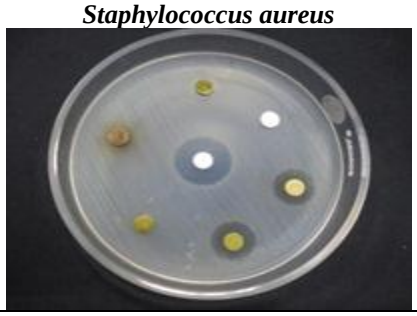
Proteus vulgaris



Salmonella paratyphi



Asian J. Pharm. Res. Vol 6, Issue 1, 7-11, 2016.

Staphylococcus aureus	***Streptococcus pneumonia***
	

CONCLUSION

Phytoconstituents present in whole plant extract of *A.paniculata* indicates their potential as a source of supply for novel medicines. Hence the crude extracts of *A.paniculata* whole plant can be used for further purification and preparation of new anti-microbial for the more resistant type of micro-organism. The above findings recommend the further investigation of *A. paniculata* to evaluate their chemical potential.

CONFLICT OF INTREST

No conflict of Interest

ACKNOWLEGEMENT

The author is grateful to the Director and Head, and also to the staff of the Rapinat Herbarium and Centre for Molecular Systematics, St. Joseph's College (Autonomous), Tiruchirappalli, Tamilnadu. He is also grateful to his Colleagues for their help and support. He thanks CSIR, New Delhi for financial support through Rajiv Gandhi National Fellowship.

REFERENCES

1. Chin Y, Balunas MJ, Chai HB, Kinghorn AD. Drug discovery from natural sources. *AAPSJ*, 8, 2006, 239- 253.
2. PerumalSamy R, Gopalakrishnakone P. Current status of herbal and their future perspectives. *Nature proceedings*, 1, 2007, 1176-1188.
3. Sangeetha J, Vijayalakshmi K. Determination of bioactive components of ethyl acetate fraction of punicagranatum Rind extract. *International journal of pharmaceutical sciences and Drug Research*, 3 (2), 2011, 116-122.
4. Vohra A, Kaur H. Chemical investigation of medicinal plant Ajugabracteosa. *Journal natural product plant Resour*., 1(1), 2011, 37-45.
5. Wu L, Gao H, Wang X, Ye J, Lu J, Liang Y. Analysis of chemical composition of *Chrysanthemum indicum* flowers by GC/MS and HPLC. *Journal of Medicinal Plants Research*, 4 (5), 2010, 421-426.
6. Sheeja K, Kuttan G. Activation of cytotoxic T lymphocyte responses and attenuation of tumour growth in vivo by *Andrographis paniculata* extract and andrographolide. *Immunopharmacol Immunotoxicol*., 29, 2007, 81-93.
7. Yoopan N, Thisoda P, Rangkadilok N, Sahasitiwat S, Pholphana N, Ruchirawat S, Satayavivad J. Cardiovascular effects of 14 deoxy 11, 12 didehydro and rographolide and *Andrographis paniculata* extracts. *Planta Med*., 73, 2007, 503-511.
8. Trivedi NP, Rawal UM, Patel BP. Hepatoprotective effect of andrographolide against Hexachlorocyclohexane, induced Oxidative injury. *Integr Cancer Ther*., 6, 2007, 271-280.
9. Kokate CK, Purohit AP and Gokhale SB. Carbohydrate and derived Products, drugs containing glycosides, drugs containing tannins, lipids and protein alkaloids. *Text book of Pharmacognosy*, 7, 2001, 428-523.
10. Khandewal KR. Practical Pharmacognocy. Nirali Prakashan, Pune, 2008, 19.
11. Peach K, Tracey MV. Modern methods of plant analysis. Springer Verlag, Berlin, 1956.
12. Treare GE, Evans WC. Pharmacognosy 17th edn. Bahiv Tinal, London, 149, 1985.
13. Kirby MM, Baur AW, Sherris JC, Tuurck M. Antibiotic susceptibility testing by a standardized single disc method. *Journal of clinical pathology*, 45, 1996, 493-496.