International Journal of Research in BioSciences
Vol. 6 Issue 4, pp. (33-39), October 2017
Available online at http://www.ijrbs.in
ISSN 2319-2844

Research Paper

Alkaloids - the healers in medicinal plants

Jaya Vikas Kurhekar
Department of Microbiology, Bharati Vidyapeeth's Dr. Patangrao Kadam Mahavidyalaya,
Sangli – 416416, Maharashtra, INDIA

(Received July 12, 2017, Accepted August 25, 2017)

Abstract

Medicine has its roots in natural plant products. Plant cells are highly sophisticated chemical factories which produce secondary metabolites like alkaloids which possess significant biological properties. They are bio-chemical interfaces between the producing plant and its surrounding environment. The relationship between human immune system and plant anti-oxidants is known and alkaloids are reported to have anti-cancerous as well as immune stimulant properties. This study investigates two easily accessible medicinal plants, *Pongamia pinnata* and *Phyllanthus niruri* with reference to their anti-microbial effects and presence of alkaloids in their leaf extracts. They exhibit good anti-microbial activity against a few bacterial pathogens causing common infections. MIC of aqueous extract of *P. pinnata* for *M. luteus* was found to be 0.0256 gm while that of *P. niruri* for *S. aureus* was found to be 0.1826 gm of plant material. The aqueous extracts of *Pongamia pinnata* and *Phyllanthus niruri* were both found effective in inhibiting the growth of ten and eight pathogens respectively, responsible for common infections. Their bio-active components were analyzed with the help of High Performance Thin Layer Chromatography (HPTLC) and showed peaks corresponding to alkaloids. Thus alkaloids can be concluded as one of the healers in plants and the natural bio resources under investigation may prove to be of significance in naturopathy and possess properties which may be further investigated.

Keywords: Secondary metabolites, alkaloids, bio-chemical interfaces, anti-cancerous, immune stimulant, anti-microbial effects, HPTLC, MIC, bio resources.

Introduction

Medicine is known to have its roots in natural plant products. The secondary metabolite is more important to serve the plant as a bio-chemical interface between the producing plant and its surrounding environment. In the early 19th century, it was realized that healing properties of medicinal plants were due to minute active ingredients. With the development of organic chemistry at the beginning of this century, extraction and fractionation techniques improved significantly. It became possible to isolate and identify many of the active constituents from plants. In 1947, "Investigation on plant antibiotics" was reported by George et al. (1947)[1]. Skinner (1955) reported the historical landmarks in the development of antibiotics[2]. In 1887, Martini first used Thymol, a simple phenol, present in the essential oils of many plants, as an antiseptic and a preservative. Some plant extracts and exudates like those of neem were observed to have some anti-fungal and anti-bacterial properties. A simplified method of evaluating dose-effect experiments and the procedure for Lethal Dose$_{50}$ (LD_{50}) determination of seed extracts was prescribed by Litchfield and Wilcoxon[3].

Quinone alkaloid, vinblastine, vincristine, dimericindole alkaloids, curcumol, citral are reported to have anti-cancerous properties[4]. Plant cells are highly sophisticated chemical factories which produce secondary metabolites including alkaloids, steroids, terpenoids, oxygen heterocyclics like flavonoids, xanthones and coumarins which possess significant biological properties[5].The

relationship between human immune system and plant anti-oxidants, especially those derived from Indian medicinal plants was reported by Devasagayam and Sainis[6]. Purine alkaloids - caffeine, theobromine and theophylline, are methyl derivatives of xanthine and co-occur in a plant. Immunostimulant, anti-inflammatory, anti-bacterial, anti-viral effects of plants is assigned to lipophilic alkylamides, polar caffeic acid derivatives, high molecular weight polysaccharide material or a combination of these. The stimulant properties are due to water-soluble alkaloids[7].

Many traditional medicinal plants have been reported to have strong antiviral activity and some are being used to treat viral infections[8]. Foods such as betel nuts and herbal teas help in defence mechanism against microbial infections. The antimicrobial, anti-oxidant, anti-mutagenic and anti-carcinogenic effects of plants have been reported. In Australia, plants have been tried out as an alternative cancer treatment.

It was reported that many medicinal plants owe their physiological activities, molecular interactions between alkaloid molecules and chemically defined components of the affected organisms, to their content of alkaloids. Alkaloids, which are plant secondary metabolites, are known to possess anti-microbial activity[9]. Antimicrobial and immune modulatory effects of medicinal plants, due to phyto-chemicals like plant alkaloids and flavonoids, have been linked to the effect on eicosanoid metabolism that influences immune functions in various ways[10]. Tiwari (2001) reported that antioxidant principles from natural resources correct the imbalance between pro-oxidant and antioxidant homeostasis, responsible for most of the diseases.

Toxic substances obtained from various plant species have been reported to control many fungal diseases of crop plants[12,13]. A number of plant species have been reported to possess some natural substances in their leaves which are toxic to many micro-organisms causing diseases. Plant produced compounds are of interest as a source of safer or more effective substitutes for synthetically produced antimicrobial agents[14]. Indiscriminate use of chemicals is not only hazardous to living beings but adversely affects the microbial population in the ecosystem. The inherent danger in the use of these chemicals has brought forth an awareness to find out alternatives like biological agents to control the disease[15]. Clinically, antimicrobial therapy is going through a crisis due to rapid development of resistance to existing antimicrobial agents[16]. On this background, a reversal to naturopathy is an alternative, which can be explored further. This study investigates two easily accessible medicinal plants, *Pongamia pinnata* and *Phyllanthus niruri* with reference to their anti-microbial effects and presence of alkaloids in their leaf extracts. Their aqueous extracts were tested for MIC to know the potency of the extracts against the most susceptible of the pathogenic isolates used. Their bio-active components were analyzed with the help of High Performance Thin Layer Chromatography (HPTLC) to check for the anti-microbial components. This was in order to check the properties and efficiency of natural bio resources under investigation which may prove to be of significance in naturopathy.

Materials and Methods

Isolation of common pathogenic isolates

Isolation of common pathogenic isolates was done from a Microbiology laboratory by collecting the samples and processing them further immediately. Isolation of the micro- organisms was carried out using corresponding media. Identification of the typical isolates was carried out using different biochemical media[17-21].The identified cultures were maintained and preserved on antibiotic assay medium (trypticase soy agar slants) for further study. Common infection causing organisms isolated included four Gram positive bacteria, eight Gram negative bacteria, one yeast and one fungal culture; *Bacillus subtilis, Staphylococcus aureus, Enterococcus faecalis, Micrococcus luteus, Escherichia coli, Klebsiella pneumoniae, Salmonella typhi, Salmonella paratyphi B, Shigella flexneri, Pseudomonas aeruginosa, Proteus vulgaris, Serratia marsescens, Candida albicans* and *Aspergillus niger.* Sensitivity of these isolates to plant extracts and antibiotics was studied using diffusion assay methods.

Detection of anti-microbial activity of the plant extracts against isolates by Agar-cup diffusion assay method[22]

Suspension of young culture of each isolate, in 0.1ml amount, was spread separately on sterile nutrient agar plates (bacterial culture) or Sabouraud's agar plates (fungal or yeast culture).Cups were aseptically made with sterile cork borers (diameter 8 mm). Aqueous and acetone extract powders were diluted 1**:**10 with the help of sterile distilled water. 0.1 ml of each extract was

aseptically added to corresponding cups and the plates refrigerated for 15 minutes. Incubation at appropriate temperatures for prescribed time was carried out. Plates were observed for zones of inhibition surrounding the cups. Diameters of the inhibition zones were recorded.

Qualitative analysis of the extracts

Qualitative analysis of the extracts was carried out with a known 1% solution of the standard and the extract.

Detection of Alkaloids[7]

Mayer's test: To 1 ml of the test solution, a few drops of Mayer's reagent (an alkaloidal precipitating reagent used for the detection of alkaloids in natural products; freshly prepared by dissolving a mixture of 1.36 g of mercuric chloride and 5.00 g of potassium iodide in 100 ml. water) were added. Development of a cream or grayish white precipitate indicated the presence of alkaloids.

Results and Discussion

Table 1: MIC of aqueous extract of *Pongamia pinnata* against *Micrococcus luteus*

S. No.	Amount (ml)	Wt. of plant Material (gm)	Growth
1	0.01	0.0032	+++
2	0.02	0.0064	+++
3	0.03	0.0096	+++
4	0.04	0.0128	+++
5	0.05	0.0160	++
6	0.06	0.0192	++
7	0.07	0.0224	+
8	0.08	0.0256	-
9	0.09	0.0288	-
10	0.10	0.0320	-
11	0.11	0.0352	-
12	0.12	0.0384	-
13	0.13	0.0416	-
14	0.14	0.0448	-
15	0.15	0.0480	-

MIC of aqueous extract of *Pongamia pinnata* against *Micrococcus luteus* is thus concluded to be **0.0256 gm** of plant material.

Table 2: MIC of aqueous extract of *Phyllanthus niruri* against *Staphylococcus aureus*

S. No.	Amount (ml)	Wt. of plant Material (gm)	Growth
1.	0.01	0.0166	+++
2.	0.02	0.0332	+++
3.	0.03	0.0498	+++
4.	0.04	0.0664	+++
5.	0.05	0.0830	+++
6.	0.06	0.0996	+++
7.	0.07	0.1162	+++
8.	0.08	0.1328	+++
9.	0.09	0.1494	+
10.	0.10	0.1660	+
11.	0.11	0.1826	-
12.	0.12	0.1992	-
13.	0.13	0.2158	-
14.	0.14	0.2324	-
15.	0.15	0.2490	-

MIC of aqueous extract of *Phyllanthus niruri* against *Staphylococcus aureus*, is thus concluded to be 0.1826 gm of plant material.

Key**:** +++refers to heavy growth of the culture, ++ refers to moderate growth of the culture, + refers to light growth of the culture

- refers to no growth of the culture.

Table 3: Results of analysis of effective plant extracts by HPTLC at 200 nm

Peak (200 nm)	Rf value	Area	Relative conc. %	Assigned component
		Pongamia pinnata		
1.	0.01	1194.3	1.36	Unknown
2.	0.13	447.4	0.51	Unknown
3.	0.21	619.4	0.70	Unknown
4.	0.26	1188.0	1.35	Unknown
5.	0.29	1158.5	1.32	Unknown
6.	0.36	58888.2	6.69	**Anthroquinones**
7.	0.40	2955.6	3.36	Unknown
8.	0.43	2764.5	3.14	Unknown
9.	0.50	16650.4	18.91	**Alkaloids**
10.	0.58	4425.8	5.03	Unknown
11.	0.63	29252.0	33.22	Unknown
12.	0.82	21506.1	24.42	Unknown
		Phyllanthus niruri		
1.	0.01	3563.1	31.34	Unknown
2.	0.21	268.6	2.36	Unknown
3.	0.27	257.5	2.26	Unknown
4.	0.30	556.3	4.89	Unknown
5.	0.34	517.6	4.55	Unknown
6.	0.40	259.4	2.28	Unknown
7.	0.44	566.6	4.98	Unknown
8.	0.52	729.6	6.42	**Alkaloids**
9.	0.63	1250.4	11.00	Unknown
10.	0.70	1747.7	15.37	Unknown
11.	0.74	1469.2	12.92	Unknown
12.	0.79	182.7	1.61	Unknown

Analysis by HPTLC at 200 nm indicated the presence of alkaloids (18.91%) in *Pongamia pinnata* while that of aqueous extract of *Phyllanthus niruri* showed peaks corresponding to alkaloids (6.42%),

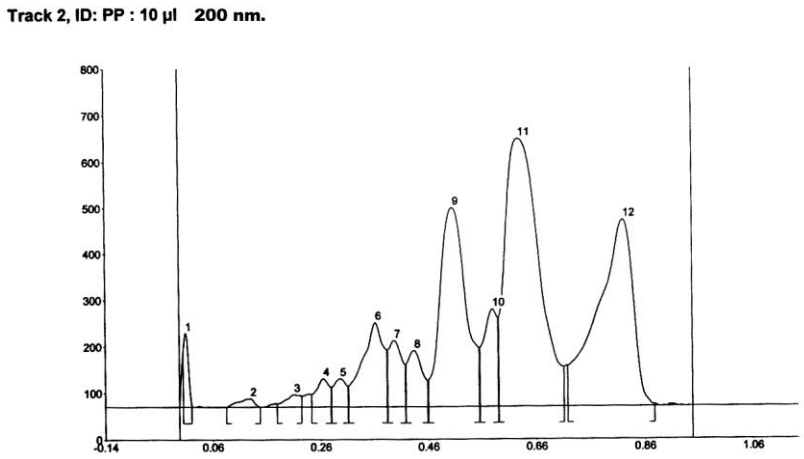

Figure 1: HPTLC chromatogram of *Pongamia pinnata* (200 nm)

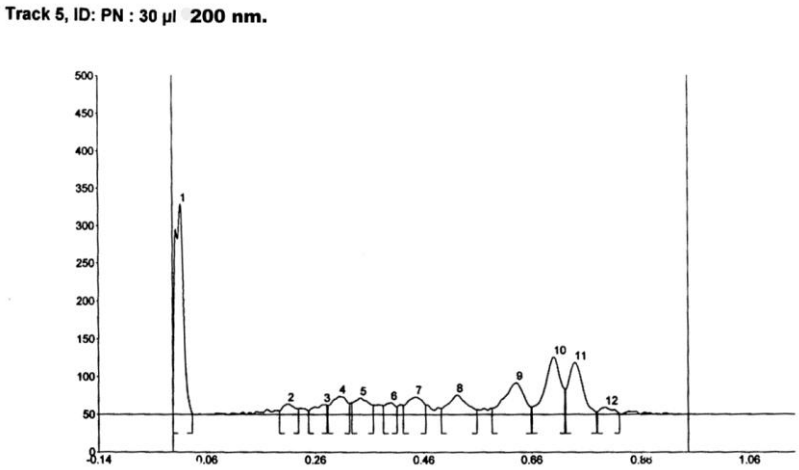

Figure 2: HPTLC chromatogram of *Phyllanthus niruri* (200 nm)

Pongamia pinnata is a huge tree, each part of which finds varied uses in the life of mankind like seeds, flowers, fruits and leaves. This plant is found to be the anti-microbially efficient. Seeds of *P. pinnata* were extracted in distilled water, acetone and 0.1 ml of each extract was tested against each isolate for antimicrobial activity. Aqueous extract (0.31 X 10^2gm%) was found effective in inhibiting the growth of Gram positive *B. subtilis*, *S. aureus, Ent. fecalis, M. luteus,* Gram negative *E. coli, Kl. pneumoniae, Sal. typhi, Sh. Flexneri, C. Albicans* and *Asp. niger*. Aqueous extract of *P. pinnata* inhibited ten of the isolates.

Commonly known as Bhui-awala, *Phyllanthus niruri* is used as an antiseptic in emergencies. 0.1 ml of each aqueous and acetone leaves extract was used against each test isolate, for antimicrobial activity. The aqueous extract (0.06 X 10^2gm%) was found effective in inhibiting the growth of Gram positive *B. subtilis, S. aureus, E. fecalis,* Gram negative *Sal. typhi, Sal. paratyphi B, P. vulgaris, Ser. Marsescens* and *C. albicans*. Aqueous extract of *P. niruri* inhibited eight of the isolates.

Akki et al. (2004) reported the effectiveness of aqueous extract of *P. pinnata* against wound pathogens, species of *Bacillus, Pseudomonas, Enterococcus, Actinomycetes* and one unidentified species[23]. Wagh et al. (2005) observed a high degree of anti-fungal and anti-bacterial activity of *P. pinnata* against *Asp. niger, Asp. fumigatus, S. aureus* and *Ps. aeruginosa*[24]. This can be attributed to the presence of 9-octadecenoic acid, methyl ester in maximum concentration. Kumar et al. (2007) reported the antimicrobial effects of *Pongamia pinnata* seeds against acne-inducing bacteria *Propionibacterium acnes* and *Staphylococcus epidermidis and* MIC and MBC of 2.5, 5.0, 2.5, 5.0 mg/ml respectively[25].

In similar studies, Akki et al. (2004) observed the wound healing activity of *P. niruri* aqueous leaf extract and reported that aqueous extract is found effective against pathogens isolated from wounds, *Pseudomonas species*, *Enterococcus species, Actinomycetal* species and two unidentified species. The alkaloid extract and sparsiflorine from *Croton bonplandianum* Baill. (Euphorbiaceae) showed moderate activity, mainly against *Pseudomonas aeruginosa*[26].

MIC of aqueous extract of *P. pinnata* (0.31 X 10^2gm%) for *M. luteus* was found to be 0.0256 gm of the plant material (Table 1).MIC of aqueous extract of *P. niruri* (0.06 X 10^2gm%) for *S. aureus* was found to be 0.1826 gm of plant material (Table 2). These values indicate the potencies of the extract against the pathogens. Moreover, the pathogens were chosen from the checking the highest efficiency of the extract in inhibiting a culture, from the size of zone of inhibition. Preliminary qualitative chemical investigation of the aqueous extract of *Pongamia pinnata* showed the presence of alkaloids, flavonoids, glucose and proteins (Table 3). Analysis by HPTLC at 200 nm indicated the probable presence of alkaloids (18.91%) (Figure 1).

Qualitative chemical investigation of the *Phyllanthus niruri* extract showed the presence of alkaloids, flavonoids, glycosides, reducing sugars and triterpenoid (Table 3). Its aqueous extract on HPTLC analysis at 200 nm probably showed peaks corresponding to alkaloids (6.42%) (Figure 2). With reports of pandrug-resistant bacteria causing untreatable infections, the need for new antibacterial

therapies is more pressing than ever. Alkaloids are a large and structurally diverse group of compounds that have served as scaffolds for important antibacterial drugs such as metronidazole and the quinolones[27]. The spread of drug resistant microorganisms is a big threat to successful therapy of microbial diseases. Therefore there is an urgent need to search new compounds characterized by diverse chemical structures and mechanisms of action. The use of different plant natural compounds as antibacterial and antifungal agents is an interesting strategy for discovering bioactive products that in the next years could become useful therapeutic tools[28].

Conclusion

Various components of medicinal plants are known to be responsible for their antimicrobial activity, one of which is alkaloid. In the present study, *Phyllanthus niruri* as well as *Pongamia pinnata* both showed the presence of alkaloids, checked analytically using a chemical test as well as through HPTLC. MICs of both plant leaf extracts indicated that small amount of the extract is required to kill pathogens. On the background of emergence of drug resistant strains of microorganisms and the cost of drugs, such natural anti microbial agents may prove useful in treating the infection. Thus alkaloids can be concluded as one of the healers in plants and the natural bio resources studied may prove to be of significance in naturopathy and possess properties which may be further investigated. Bioactive molecules may prove to be good therapeutic tools.

References

1. George M., Venkataraman P.R. and Pandalal K.M., Investigation on Plant Antibiotics. J. of Scientific and Ind. Res., 6 B: 342- 346 **(1947)**

2. Skinner F.A., Antibiotics, In: Modern Methods of Plant Analysis, eds. Paech K., Tracey M.V., Band Dritter, Springer-Verlag, Berlin. Gottingen. Heidelberg, III: 40 **(1955)**

3. Litchfield J.T. and Wilcoxon F.J., A Simplified Method of Evaluating Dose-Effect Experiments. J. Pharmacol. Expt. Therap., 96: 99-113 **(1949)**

4. Mondal P. and Mukherjee P.K., Notes of Ethno-botany of Keonjhar dist., Orissa, J. Econ. Tax. Bot. Addl. Ser., 10: 7-18 **(1992)**

5. Baskar R.G. and Chezhiyan N., Plant compound and Chemotherapy – Role of Biotechnology in Medicinal and Aromatic Plants. Special Volume on Diseases, Ukaaz Publication, Hyderabad, VI: 124 **(2002)**

6. Devasagayam T.P.A. and Sainis K.B., Immune System and Anti-oxidants, especially those derived from Indian Medicinal Plants. Ind. J. Exp. Biol., 40: 643 **(2002)**

7. Kokate C.K., Purohit A.P. and Gokhale S.B., Pharmacognosy, 17th edition, Nirali Prakashan, Pune: 58, 197, 445, 586-588 **(2001)**

8. Jassim S.A.A. and Naji M.A., Novel Antiviral Agents : A Medicinal Plant Perspective, J. App. Microbiol., 95: 412-427 **(2003)**

9. Cowan, M.M., Plant Products as Antimicrobial Agents. Clinical Microbiological Reviews, 12: 564-582 **(1999)**

10. Ali M., Thomson M. and Afzal M., Garlic and Onions: Their Effect on Eicosanoid Metabolism and its Clinical Relevance. Prostaglandins Leukot Essential fatty acids, 62:5 **(2000)**

11. Tiwari A.K., Imbalance in Anti-Oxidant Defence and Human Disease, Multiple Approach of Natural Antioxidant Therapy. Curr. Sci., 81: 1179 **(2001)**

12. Chary M.P., Reddy E.J.A. and Reddy S.M., Screening of Indigenous Plants for their Anti-fungal Principles. Pesticides, 18: 17-18 **(1984)**

13. Singh R.K. and Dwivedi R.S., Fungicidal Properties of Neem and Babulgum against *Sclerotiumralfsii*. Acta Bot. Indica, 18: 260-262 **(1990)**

14. Heisy R.M., Bernadette and Gorham K., Antimicrobial Effects of Plant Extracts on *Streptococcus mutans*, *Candida albicans, Trichophytonrubrum* and other Micro-organisms. Letters in Applied Microbiology, 14: 136-139 **(1992)**

15. Ansari M.M., Control of Sheath Blight of Rice by Plant Extracts. Ind. Phytopath., 48: 268-270 **(1995)**

16. Bonjar S.G.H., Nik A.K., Heydari M.R., Ghasemzadeh, M.H., Farrokhi P.R., Moein M.R., Mansouri S. and Foroumadi A., Anti-pseudomonas and Anti-bacilli Activity of Some Medicinal Plants of Iran. DARU, 11: 157-163 **(2003)**

17. Saxena K.P., A Brief Course in Algae and Fungi, Prakashan Kendra, Lucknow: 97-102 **(1969)**

18. Frobisher Hinsdill, Crabtree and Goodheart, Fundamentals of Microbiology. Ninth edition, Toppan Company, Japan: 467-570 **(1974)**

19. Ananthanarayan R. and Paniker J.C.K., Text book of Microbiology, 3rd edition, Orient Longman. Madras: 183-310 **(1986)**

20. Dey N.C. and Dey T.K., A Text Book of Medical Bacteriology and Microbiology. 16th edition, New Control Book Agency, Calcutta: 25.1 - 32.1 **(1998)**

21. Gupte S., Short Text Book of Medical Microbiology. Jaypee publication: 221-323, (1998)

22. Finegold, S.M. and Baron, E.J., Diagnostic Microbiology, 7th Edn., The C.V. Mosby Company, St. Louis: 176 **(1986)**

23. Akki, K.S., Hukkeri V.I., Karadi R.V., Savadi R.V. and Manohara K.P., Wound Healing Activity of *Eucalyptus globulus* Leaf Extract, Ind. J. Nat. Prod., 20: 19-22 **(2004)**

24. Wagh P., Rai M., Marta C. and Teixeira D., Chemical Composition and Anti-microbial Activity of Oils extracted from *Trigonellafoenum-graecum* L. and *Pongamiapinnata* L. Pierre. National Conference on Bioactive Compounds: New Frontiers and Therapeutic Usage, Nanded:14, **(2005)**

25. Kumar G.S., Jayaveera K.N., Ashok Kumar C.K., Umachigi P.S., Swamy V.B.M. and Kishore Kumar D.V., Antimicrobial Effects of Indian Medicinal Plants Against Acne-Inducing Bacteria. Tropical J. of Pharma. Res., 6: 717-723 **(2007)**

26. Burgos J. Barua, Flores-Giubi M.E., Bazan D., Ferro E. and Alvarenga N.L., Antibacterial activity of the alkaloid extract and isolated compounds from *croton bonplandianum* baill. (euphorbiaceae) rev. Bras. Plantas med. 17 (4), supl.2 botucatu **(2015)**

27. Cushnie T., Cushnie B. and Lamb A., Alkaloids: An overview of their antibacterial, antibiotic-enhancing and antivirulence activities. Int. J. Antimicro. Agen., 44(5), 377 -386 **(2014)**

28. Dianella S., Plant Derived Antimicrobial Compounds – An alternative to antibiotics. Future Microbiol., 7(8): 979-990 **(2012)**