ORIGINAL ARTICLE

Evaluation of antimicrobial and analgesic activities of *Aporosa lindleyana* (*euphorbiaceae*) bark extract

Lingadahalli P. Srikrishna[1], Hosadu M. Vagdevi[1], Basavanakote M. Basavaraja[1], Vijayavittala P. Vaidya[2]
[1]Departments of PG Studies & Research in Industrial Chemistry and [2]PG Studies & Research in Chemistry, Kuvempu University, Shankaraghatta - 577 451, India

The present study was designed to evaluate the antimicrobial and analgesic activities of pet ether, chloroform, methanol and water extracts of the bark of *Aporosa lindleyana* plant belonging to Euphorbiaceae family. Antibacterial activity has been carried out using cup-plate method and reported in millimeters. All the extracts showed moderate to very good activity against bacteria *Bacillus subtilus, Escherichia coli, Staphylococcus aureus* and *Klebsiella pneumonia* and compared with the standard drug Tetracycline. Antifungal activity has been studied on the organisms such as *Pencilleum chrysozenous, Candida albicans, Aspergilus niger* and *Trichoderma vridar* and compared with the standard drug Fluconazole. Analgesic activity has been carried out on Swiss albino male mice by abdominal constriction method. All the extracts showed moderate analgesic activity.

Key words: *Aporosa lindleyana*, antimicrobial activity, analgesic activity, *euphorbiaceae*

INTRODUCTION

Medicinal plants occupied an important position in the socio-cultural, spiritual and medicinal arena of rural people of India. The Indian system of medicines, i.e. Ayurveda, Siddha, Unani and Homeopathic system predominantly use plant-based raw materials in most of their preparations and formulations. The World Health Organization (WHO) estimated that, 80% of the populations of developing countries rely on traditional medicines, mostly plant drugs, for their primary health care. Demand for medicinal plants is increasing in both developing and developed countries due to growing recognition of natural products, being non-narcotic, having less side-effects, easy availability and at affordable price.

Nature has bestowed upon us a very rich botanical wealth and a large number of diverse types of plants grow wild in different parts of our country. In India, the use of different parts of medicinal plants to cure specific ailments was in practice from ancient times.[1] India is rich in medicinal plant diversity. All known types of agro-climatic and ecologic conditions exist within India, and is rich in all three levels of biodiversity, as species diversity, genetic diversity and habitat diversity.[2] Approximately 20% of the world plants have been explored in pharmacological and biological test diseases. The clinical efficacy of many existing antibiotics is being threatened by the emergence of multidrug-resistant pathogens.[3] Bacterial and fungal have evolved numerous defense mechanisms against antimicrobial agents and resistance to old and newly produced drugs. The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the screening of several medicinal plants for their potential antimicrobial activity.[4,5] There are several reports in the literature regarding the antimicrobial activity of crude extracts prepared form plants.[6-9] *Euphorbiaceae* family is the sixth largest family among flowering plants. The genus *Euphorbia* of this family, alone account for one sixth of the whole group of flowering plants, with about 1000 species documented.[10] Many of these species have been the subject of chemical and pharmacological investigation. Other *Euphorbia* species were reported to have reported to have antibacterial activity.[11-13]

Escherichia coli causes septicemias and can infect the gall bladder, meninges, surgical wounds, skin lesions and the lungs especially in debilitate and immunodeficient patients.[14,15] There are various reports in the literature regarding characterization of medicinal plant extracts that may inhibit the bacteria. The potential of *Mesua ferrea* flowers has been reported[16] and organic solvent extracts of *P. commutate* inhibitory activity against *E. coli K. pneumonia*.[17]

Aporosa lindleyana plant is commonly known as salle (in Kannada). The tree is frequently found in the Malnad

For correspondence: Dr. Hosadu M. Vagdevi, Department of PG Studies and Research in Industrial Chemistry, Kuvempu University, Shankaraghatta - 577 451, India. E-mail: vagdevihm@gmail.com
Received: 14-05-08; **Accepted:** 20-06-2008

region of Karnataka state. The plant is used as a traditional medicine for heeling skin diseases. The present work aims in evaluating the antimicrobial and analgesic activities of *aporosa lindleyana* plant.

MATERIALS AND METHODS

Plant Materials

Fresh bark of *Aporosa lindleyana* plant was collected from Koppa taluk of Chickmagalur district of Karnataka state during the month of May, 2007. The specimen herbarium, (KU/SD/TI 545) was identified by a taxonomist, Dept. of Applied Botany, Kuvempu University, Shankaraghatta.

Bark Extract of *Aporosa lindleyana*

The bark of the plant was shade dried and powdered. The powdered bark was extracted with solvents of increasing polarities, such as, petroleum ether, chloroform, methanol and water by hot soxhlet extraction process for 40 cycles. The extracts were filtered and solvents were evaporated by rotary evaporator at reduced pressure. The obtained extracts were used for *in vitro* antibacterial, antifungal and analgesic studies.

Preparation of Culture Media

Nutrient Agar Media

The nutrient broth media was prepared by dissolving nutrient agar (14 gm) in distilled water (500 ml), the pH of the solution was adjusted to 7.4 and then sterilized for 15 min at 15 lb pressure in an autoclave.

Antimicrobial Activity

The cup-plate method was used for evaluating antimicrobial activity.[18] The antibacterial activity of the crude extracts was studied against four bacterial strains such as *Staphylococcus aureus, Bacillus subtilis, Klebsiella pneumoniae,* and *Escherichia coli.* The plates were incubated at 37^0 C temperature for 48h. The crude extracts were tested at a concentration of 10 mg/ml in dimethyl formamide against all the organisms. Tetracycline (10 µg/100 µl) was used as standard for comparison of antibacterial activity. The antifungal activity of the crude extracts was screened by the potato dextrose agar well diffusion method[19] against four fungal strains such as *Aspergillus niger, Candida albicans, Pencillium chrysozenous* and *Trichoderma vridar*. The crude extracts were tested at a concentration of 10 mg/ml in dimethyl formamide against all the four organisms. Fluconazole (10 µg/100 µl) was used as standard for comparison of antifungal activity. The plates were incubated at 25^0 C for 72h. The results are tabulated in Table 1.

Analgesic Activity

Animals

Adult Swiss albino mice (25-30 g, 6 animals per group) were used for the abdominal constriction method. They were housed individually in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 25-27°C and relative humidity of 30-70%.

This method was based on acetic acid induced writhings in mice.[20] Male Swiss albino mice were procured from Virus Diagnostic Laboratory, Shimoga. Five groups of four mice each (22-35 g) were selected and 0.6% acetic acid (dose 10 ml/kg) was injected intraperitoneally. The numbers of writhes were counted for 20 min, after 5 min of injection of acetic acid to each mice. This reading was taken as control. Next day the same groups of mice were used for evaluating analgesic activity. Each group was administered orally with the suspension of test extract in 0.1% Tween-80 solution at the dose of 100 mg/kg body weight of the animal 1 h before injection of acetic acid. After 5 min, the mice were observed for the number of writhes for the duration of 20 min. The mean value for each group was calculated and compared with the control. Acetyl salicylic acid was used as standard for comparison of analgesic activity and the results are recorded in [Table 2]**.** Percent protection is calculated using the formula, $(1\text{-}V_c/V_t) \times 100$.

RESULTS AND DISCUSSION

The antimicrobial results reveal that, [Table 1] the activity of the crude extracts of *Aporosa lindleyana* plant is encouraging. All the four tested bacterial organisms are almost equipotent with that of standard. The pet ether extract showed considerable activity towards all the four fungal organisms. Methanol extract showed very good analgesic activity and

Table 1: Antimicrobial activity of bark extract of *Aporosa lindleyana*

Extracts	Zone of inhibition (in mm) Mean±SE				Zone of inhibition (in mm) Mean±SE			
	B. subtilis	*E. coli*	*S. aureus*	*K. pneumoniae*	*P. chrysozenous*	*C. albicans*	*A. niger*	*T. vridar*
PE	27.30±0.12	18.37±0.23	18.80±0.16	16.70±0.06	14.47±0.44	14.10±0.56	13.13±0.27	12.27±0.16
$CHCl_3$	18.23±0.23	16.77±0.58	22.27±0.44	17.50±0.22	6.40±0.18	16.30±0.31	11.30±0.18	13.23±0.21
Methanol	16.13±0.09	17.43±0.30	17.50±0.31	16.83±0.29	11.57±0.22	0.97±0.41	10.73±0.24	9.30±0.54
Water	0.90±0.36	0.87±0.32	0.87±0.24	1.03±0.36	0.77±0.23	0.80±0.54	0.70±0.16	0.63±0.13
Standard	30.40±0.08	20.40±0.37	32.33±0.36	20.33±0.38	19.37±0.56	25.27±1.76	30.40±0.33	25.47±0.36

The value of each constituents consisted of Mean ± SE of 03 replicates. Value is significantly different when $P< 0.05$. PE-Petroleum ether, $CHCl_3$-Chloroform, Standard antibacterial-Tetracycline, antifungal- Flucanozole. *B. Subtilis: Bacillus subtilis, E. coli: Escherichia coli, S. aureus: Staphylococcus aureus, K. pneumoniae: Klebsiella pneumoniae, P. chrysozenous: Pencillium chrysozenous, C. albicans: Candida albicans, A. niger: Aspergillus niger, T. vridar: Trichoderma vridar*

Table 2: Analgesic activity of *Aporosa lindleyana* bark extract

Extracts	Dose mg/kg	Mean no. of writhing		% Protection
		Before injection of drug	After injection of drug	
Std	100	23.83±2.31	08.83±0.94	62.93
Pet ether	100	28.00±1.26	18.16±1.47	35.14
Chloroform	100	19.66±1.99	11.83±2.16	39.83
Methanol	100	23.00±2.89	11.16±1.07	51.45

Values are in mean ± SEM
Index for analgesic activity study
Method : Acetic acid induced writhing (acetic acid-0.6% concentration)
Animal : Albino mice.
No. of animals per group : 6(25-30g)
Route of administration : IP (Intraperitoneally)
Standard drug used : Acetyl salicylic acid (Aspirin)
SEM : Standard Error Mean.

pet ether extract showed considerable analgesic activity.

CONCLUSIONS

Herbs are an integral part of nature. Plants contain natural substance that can promote health. Antimicrobial activity and analgesic activity of bark extracts of *Aporosa lindleyana* plant is helpful in treating various kinds of diseases in future days. The antimicrobial and analgesic activities of this plant highlighted the importance of the extracts in traditional preparations.

ACKNOWLEDGEMENT

Authors are thankful to Dr. H. M. Prakash, Department of Applied Botany, Kuvempu University, for identification of plant. Authors are also thankful to Mr. B. G. Harish, Department of Biotechnology and Mr.C. Chandrashekar, Department of Chemistry, Kuvempu University, Shankaraghatta for their help in carrying out antimicrobial activities.

REFERENCES

1. Bhattacharjee SK. Handbook of Medicinal Plants. Jaipur, India: Pointer Pub; 1998. p. 1-6.
2. Zafar M, Iqbal A, Faiz M. Indian medicinal plants: A potential source for anticandidal drugs. J Ethnopharmacol 1999;37:237-42.
3. Bandow JE, Brotz H, Leichert LI, Labischinski H, Hecker M. Proteomic approach to understanding antibiotic action. Antimicrob Agents Chemother 2003;47:948-55.
4. Colombo ML, Bosisio E. Pharmacological activities of *Chelidonium majus* (Papaveraceae). Pharmacol Res 1996;33:127-34.
5. Scazzocchio F, Comets MF, Tomassini L, Palmery M. Antibacterial activity of *Hydrastis canadensis* extract and it's major isolated alkaloids. Planta Med 2001;67:561-3.
6. El-Seedi HR, Ohara T, Sata N, Nishiyama S. Antimicrobial terpenoids from *Eupatorium glutinosum* (Asteraceae). J Ethnopharmacol 2002;81:293-6.
7. Rojas R, Bustamante B, Bauer J, Fernandez I, Alban J, Lock O, Antimicrobial activity of selected Peruvian medicinal plants. J Ethnopharmacol 2003;88:199-204.
8. Duraipandiyan V, Ayyanar M, Ignacimuthu S. Antimicrobial activity of some ethnomedicinal plants used by Paliyar tribe from Tamil Nadu, India. BMC Complement Altern Med 2006;6:35-41.
9. Parekh J, Chanda S. *In vitro* antimicrobial activity and phytochemical analysis of some Indian medicinal plants. Turk J Biol 2007a;31:53-8.
10. Mabberley DJ. *The Plant Book*. Cambridge, UK: Cambridge University Press; 1987. p. 218.
11. Khan NH, Rahman M, Nur-e-Kamal MS. Antibacterial activity of *Euphorbia thymifolia.* Indian J Med Res 1988;87:395-7.
12. Vijaya K, Ananthan S, Nalini R. Antibacterial effect of theaflavin, polyphenon 60 (*Camellia sinensis*) and *Euphorbia hirta Shigella:* A cell culture study. J Ethnopharmacol 1995;49:115-8.
13. Sutthivaiyakit S, Thapsut M, Prachayasittikul V. Constituents and bioactivity of the tubers of *Euphorbia sessiliflora*. Phytochemistry 2000;53:947-50.
14. Parekh J, Chanda S. *In vitro* activity of methanol extract of *Woodfordia fruticosa Kurz*, flower (Lythraceae). Braz J Micro 2007;38:204-7.
15. Black JG, Microbiology: Principles and application. Prentice Hall NJ: 1996. p. 260.
16. Mazumder R, Dastidar SG, Basu SP, Mazumder A, Singh SK. Antibacterial potentiality of *Mesua ferrea* flowers. Phytother 2004;18:824-6.
17. Ilhan S, Savaroglu F, Colak F, Filik Iscen C, Erdemgil FZ. Antimicrobial activity of *Palustriella commutate* (Hedw.) Ochyra extracts (Bryophyta). Turk J Biol 2006;30:149-52.
18. Saundane AR, Rudresh K, Satyanarayan ND, Hiremath. Pharmacological screening of 6H, 11H-indolo {3, 2-c} isoquinolin-5-ones and their derivatives. Indian J Pharm Sci 1998;60:379.
19. Parekh J, Chanda S. *In vitro* antimicrobial activity and phytochemical analysis of some Indian medicinal plants. Turk J Biol 2007a;31:53-8.
20. Vagdevi HM, Vaidya VP. Indian Journal of Heterocyclic Chemistry 10, April 2001. p. 253.
21. Kinghorn AD, Evans FJ. Skin irritants of Euphorbia fortissima. J Pharm Pharmacol 1975;27:329-33.
22. Upadhyay RR, Sater AM, Moinzadeh F, Bunakdari A, Sedehi F, Samin R. Tumor promoting activity of *Euphorbia striatella* (Boiss) and skin irritant activity of some *Euphorbia* species. *Neoplasma* 1984;31:347-50.
23. El-Hafiz MA, Weinger B, Quirion JC, Anton R. Ketoalcohols, lignans and coumarins from Chiococca alba. Phytochemistry 1991;30:2029.
24. Gundidza M, Kufa A. Skin irritant and tumour promoting extract from the latex of *Euphorbia bougheii*. Cent Afr J Med 1993;39: 56-60.

Source of Support: Nil, **Conflict of Interest:** None declared.