Indian Journal of Natural Products and Resources
Vol. 1(1), March 2010, pp. 17-21

Assessment of antimicrobial and antioxidant activities of *Dendrocnide sinuata* (Blume) Chew leaves–A medicinal plant used by ethnic communities of North East India

Bhaben Tanti[1,*], Alak Kumar Buragohain[2], Lisha Gurung[1], Debashree Kakati[1], Ajoy Kumar Das[1] and Sailendra Prasad Borah[1]

[1]Cytogenetics and Plant Breeding Laboratory, Department of Botany, Gauhati University, Guwahati-781 014, Assam, India
[2]Department of Molecular Biology and Biotechnology, Tezpur University, Tezpur-784 028, Assam, India

Received 18 August 2008; Accepted 30 July 2009

Traditional medicine plays an important role in the primary health care in India. *Dendrocnide sinuata* (Blume) Chew has been used as medicine for curing diseases by different tribal communities of North East India. An ethno-medicinal study was done among few tribal communities of this region through questionnaires in consultations with the tribal practitioners and has resulted in the documentation of various uses of the plant for curing diverse form of ailments. Further, *in vitro* study was carried out to investigate its antimicrobial and antioxidant properties from the leaf extract of the plant. The methanol and aqueous extracts of leaves were tested for their antimicrobial activity against three Gram-positive bacteria, three Gram-negative bacteria, one yeast species using Agar diffusion method and for their antioxidant activity using scavenging activity of DPPH (1,1-diphenyl-2-picrylhydrozyl) radical method. Antimicrobial activity was observed against Gram-negative bacteria only. The highest antimicrobial activity was exhibited by the 75 and 100% methanolic extracts but no extract showed any antifungal activity against *Candida albicans* used in the study. The methanolic leaf extracts of 75 µg/ml and 100µg/ml concentrations also exhibited high free radical scavenging activity. The phytochemical screening demonstrated the presence of different types of compounds like terpenoids, tannins, flavonoids and others, which could be responsible for the antimicrobial and antioxidant activities.

Keywords: Antimicrobial, Antioxidant, *Dendrocnide sinuata*, Traditional medicine; North East India.

IPC code; Int. cl.[8]—A61K 36/00, A61P 31/00, A61P 39/06

Introduction

Drugs of plant origin remain an important source to combat serious diseases, especially in developing countries. Approximately 60-80% of the world's populations still depend on traditional medicines for the treatment of common illnesses[1]. Traditional knowledge of using medicinal plants in North East India has been both explicit and tacit that has been codified into words or transferred from one generation to other through ages; thereby suggesting a sense of common ownership amongst different communities[2,3]. Nevertheless, little scientific research has been done to investigate the plants of this region used in herbal medicine and various problems associated with them. Therefore, during the present investigation, the background information on traditional uses of *Dendrocnide sinuata* (Blume) Chew was collected from a few ethnic tribes of North East India.

*Correspondent author:
E-mail: bt53@rediffmail.com; btanti@gauhati.ac.in
Phone: 094012-03977 (Mob.)

Dendrocnide sinuata (Blume) Chew has been widely used for a variety of diseases by the local tribes of North East India. The plant belonging to the family *Urticaceae* is a perennial herb, dull-green in colour, armed with minute rigid hairs or prickles, which transmit a venomous fluid when pressed. The stem is obtusely 4-angled, branching, up to 1.5m high, arising from a branching root, with fleshy shoots and many fibres. The leaves are opposite, petiolate, cordate, lanceolate, spreading, conspicuously acuminate, coarsely and acutely serrate, the point entire, armed with stings, 7-10cm long and about 1.5cm wide. The flowres are small, green, clustered, axillary, interrupted spikes and longer than the petioles (Fig. 1).

The aim of this work was to investigate the ethnomedicinal uses particularly antimicrobial and antioxidant activities of *D. sinuata* leaves which are traditionally used by different tribal communities in North East India. In recent years, prevalence of infections has increased to a great extent and resistance against antibiotics has become an ever-

Fig.1— Natural habitat of *Dendrocnide sinuata*

increasing therapeutic problem[4]. Natural products of higher plants may give a new source of antimicrobial agents[5,6]. In the last few years, interest in the antioxidant activity of plant extracts has increased tremendously which is very important due to the fact that free radicals e.g. reactive oxygen species (ROS) can be responsible for various diseases like heart diseases, stroke, arteriosclerosis and cancer as well as for ageing process[7-9]. Many antioxidants are plant based and play vital role in protecting plants that are exposed to strong sunlight and live under oxygen stress[10]. In the present study, methanolic crude extract and hot aqueous extracts prepared from leaves has been studied for their antimicrobial activity by means of the Agar diffusion method and antioxidant activity using scavenging activity of DPPH (1,1-diphenyl-2-picrylhydrozyl) radical method. Furthermore, a phytochemical screening of the methanolic extract was also performed.

Materials and Methods

Ethno-medicinal study

A survey was conducted to collect information on the medicinal uses of *D. sinuata* among few of the tribal communities, viz. Nishi (Arunachal Pradesh), Apatani (Arunachal Pradesh), Addibasi (Assam), Karbis/Dimasa (Assam), Khashi (Meghalaya), Reang (Tripura) of North East India. Information was gathered from the village chiefs (Gaon Burahs), medicine man and even local men and women using prepared questionnaires. During the study, prior information consent (PIC) of community has been taken from the knowledge providers. Analysis of data was made with the help of group discussions among different age classes of villagers that include both the genders of the society. A total of 440 villagers (308 men and 132 women) participated in the study, but only 242 (~55% of the 440) provided information for all the methods of data collection. The final sample was almost evenly split between men (n = 134, or 55.3%) and women (n =108, or 44.6%). Information was recorded with regards to the plant parts used, process of preparation of medicine i. e. individually or in combination with other plants, mode of application and doses for the treatment of a particular disease or diseases.

Collection of plant material

For *in vitro* study, whole plant of *D. sinuata* was collected from and around Guwahati, Assam, India. The herbarium specimen was made following standard herbarium techniques[11], identified with the help of references and herbarium specimen of Botanical Survey of India, Shillong (India) and the voucher specimen No. 067 of the study has been deposited in the herbarium of the Department of Botany, Gauhati University (Assam), India.

Extraction of plant materials

Leaves of the *D. sinuata* were dried under shade and powdered using mortar and pestle. The powdered plant material (10g) was extracted with 400 ml methanol (CH_3OH) by using a Soxhlet apparatus for 8h. The residue was dried over night and then extracted with 250 ml distilled H_2O using a shaking water-bath at 70ºC for 2h. The extraction with water was repeated thrice. The water filtrates were mixed together. The final methanolic and aqueous extracts were filtered and evaporated using a rotary evaporator and freeze dryer, respectively to obtain the crude dried extract. The dried extracts were stored at 20ºC until used.

Test organisms

The microorganisms used as test organisms were obtained from Institute of Microbial Technology (IMTECH), Chandigarh, India. Three Gram-positive bacteria, viz. *Staphylococcus aureus* (MTCC 96), *Bacillus subtilis* (MTCC 1789), *Micrococcus luteus* (MTCC 106); three Gram-negative bacteria, viz. *Escherichia coli* (MTCC 1687), *Pseudomonas aeruginosa* (MTCC 1034), *Enterobacter aerogenes* (MTCC 111) and one yeast species, viz. *Candida albicans* (MTCC 183). The bacterial strains were maintained on nutrient agar (Difco) slants and the yeast strains on YEPD slants at 4ºC and subcultured for 24h before use.

Antimicrobial assay

The disc-diffusion assay was used to determine the antimicrobial activity of the extracts under investigation. Nutrient agar (Difco) was prepared as

per the manufacture's protocol by dissolving of 27g/l in distilled water. The sterile nutrient agar was inoculated with microbial cells (200µl of microbial cell suspension in 20 ml agar medium) and poured into sterile petridishes. Sterile filter paper discs of 6mm diameter (Schleicher and Schuell, ref. No. 10321260, lot. DG0274-1) were impregnated with 20 µl of the extract solution (equivalent to 4 mg of the dried extract). The paper discs were allowed to evaporate and after that placed on the surface of the inoculated agar plates. Plates were kept for 2h in refrigerator to enable pre-diffusion of the extracts into the agar. Then, the plates were incubated overnight (18h) at 37ºC. In contrast, *M. luteus* (MTCC 106) was incubated at room temperature for 48h and *C. albicans* (MTCC 183) was incubated at 28ºC for 48h. Ampicillin, gentamicin and amphotericin B were used as positive control. Negative controls were performed using paper discs loaded with 20 ml of organic solvents (chloroform and methanol). At the end of the incubation period the antimicrobial activity was evaluated by measuring the inhibition zones (diam. of inhibition zone plus diam. of the disc). An inhibition zone of 14mm or more was considered as high antimicrobial activity[12].

Determination of antioxidant activity

In order to measure antioxidant activity, DPPH free radical scavenging assay was used. This assay measures the free radical scavenging capacity of the extracts under investigation. DPPH is a molecule containing a stable free radical. In the presence of an antioxidant, which can donate an electron to DPPH, the purple color which is typical for free DPPH radical decays and the change in absorbency at 517nm was measured using Backman DU-530 spectrophotometer[13]. The methanolic extract was redissolved in methanol and various concentrations (10, 25, 50, 75 and 100µg/ml) of extract were used. The assay mixture contained in total volume of 1 ml, 500µl of the extract, 125µl prepared DPPH and 375µl solvent (methanol). After 30 min of incubation at 25ºC, the decrease in absorbance was measured at 517nm on spectrophotometer. The radical scavenging activity was calculated from the equation:

Radical scavenging activity (%) = $(Abs_{control} - Abs_{sample})/Abs_{control} \times 100$

Phytochemical screening of the methanolic extract

The screening of chemical constituents was carried out with the methanol extract using chemical methods and thin-layer chromatography (TLC) according to the methodology described by Wagner and Bladt[14].

Results and Discussion

Some of the ethnomedicinal uses of the plant by few ethnic tribal communities of North East India have been listed in Table 1. For curing diverse form of ailments, leaf of the plant is used by most of the tribal communities of this region[15,16]. The present study notes ethnomedicinal, antimicrobial and antioxidant activities of *D. sinuata* used as traditional medicine by most of the ethnic tribes of North East India.

The antimicrobial activity of specific concentrations of hot aqueous and methanol extract of

Table 1— *Dendrocnide sinuata* used by few ethnic tribes of the North East India

Name of the ethnic tribes	Plant part(s) used	Ethnomedical preparation and use
Nishi (Arunachal Pradesh)	Root	(i) Root pounded, warmed and a paste is made and applied against swollen muscles, injury and itching; (ii) Leaves are mixed with leaves of *Stephania glabra* (2:1) and boiled with water. 1-2 teaspoonful of decoction is administered as a remedy for fever, malaria and while feeling hot.
Apatani (Arunachal Pradesh)	Leaves	(i) Young leaves are boiled and the decoction is administered in case of urinary disorder or reddish urine of male and female; (ii) Leaves are boiled and the decoction is administered as a remedy for dysentery.
Adibasi (Assam)	Leaves and roots	(i)Paste is applied locally on painful boil; (ii) A small piece of root mixed with a small piece of root of *Carica papaya* Linn., fruits (3 nos.) of *Piper nigrum* Linn. and 2-3 small pieces of root of *Solanum nigrum* Linn., made into paste and is boiled with about 250 ml of water. The extract is prescribed usually to women to regularize menstrual cycle (1-2 teaspoonful/day for 3-4 days).
Karbis/Dimasa (Assam)	Leaves	Juice of leaves is used in chronic fever.
Khashi (Meghalaya)	Roots/Leaves	Root and leaf paste is applied on swelling and blind abscesses.
Reang (Tripura)	Leaves	Few young leaves are fried with edible oil and taken along with the rice for 7 days for remedies of hypersensitivity.

the investigated plant is shown in Table 2. The antimicrobial activity of the plant extracts was exhibited mainly against the Gram-negative bacteria. None of the extracts showed any activity against Gram-positive bacteria. Among the investigated extracts the methanol extract exhibited the highest antimicrobial effect. The most pronounced activity with inhibition zones more than 14mm was shown by the methanol extract of 75 and 100%. The majority of the hot aqueous extracts of the antimicrobial active plants did not express any activity or exhibited only low activity. The reasons accounting for the higher antimicrobial activity of methanol extracts might be firstly due to the nature of biological active components (terpenoids, tannins, flavonoids, etc), which might be enhanced in the presence of methanol and secondly, the stronger extraction capacity of methanol might have produced a greater number of active constituents responsible for antimicrobial activity. It was observed that no extract possesses antifungal activity against *C. albicans* (MTCC 183). The results of the phytochemical screening of the investigated methanolic extracts showed the presence of different types of active constituents like flavonoids, terpenoids, tannins, etc.

The methanol extract of the investigated plant showed a high effective free radical scavenging in the DPPH assay. The extract exhibited a remarkable antioxidant effect at low concentrations. When different concentrations of standard reference compounds (Gallic acid) were added to 1 ml of solution of DPPH (0.2 mM), inhibition of DPPH radical was observed for all concentrations of standard compound used (Table 3). 85% and above inhibition of DPPH radicals was observed in case of 10µg/ml gallic acid used. The free radical inhibition potential of any compound depends on its ability to donate hydrogen or free electrons.

Table 2—Antimicrobial activity of *Dendrocnide sinuata*

Plant extract	Concentration (%)	Microbial strains tested						
		S.a	B.s.	M.l.	E.c.	P.a.	E.a.	C.a.
Methanol								
	10	-	-	-	11	9	11	-
	25	-	-	-	12	11	11	-
	50	-	-	-	13	9	14	-
	75	-	-	-	14	11	14	-
	100	-	-	-	16	14	20	-
Hot Aqueous								
	10	-	-	-	-	-	-	-
	25	-	-	-	-	-	-	-
	50	-	-	-	-	-	-	-
	75	-	-	-	8	-	-	-
	100	-	-	-	9	-	10	-
Ampicillin (10µg/disc)		15	17	17	N.T.	N.T.	N.T.	N.T.
Gentamicin (10µg/disc)		N.T.	N.T.	N.T.	25	26	30	N.T.
Amphotericin (10µg/disc)		N.T.	N.T.	N.T.	N.T.	N.T	N. T.	10

S.a, *Staphylococcus aureus* (MTCC 96); B.s., *Bacillus subtilis* (MTCC 1789); M.l., *Micrococus luteus* (MTCC 106); E.c., *Escherichia coli* (MTCC 1687); P.a., *Pseudomonas aeruginosa* (MTCC 1034); E.a., *Enterobacter aerogenes* (MTCC 111); C.a., *Candida albicans* (MTCC 183); -, no activity; N.T. , not tested; Inhibition zones including the diameter of the paper disc (6mm).

Table 3—Free radical scavenging activity of the gallic acid and methanolic plant extract (PE) on DPPH-Free Radical
(Data are expressed as mean ± SD, where n = 5)

Concentration (µg/ml)	DPPH free radical scavenging activity (%)	
	PE	Gallic acid
10	07.16 ± 0.13	89.31±0.18[a]
25	19.29 ± 0.23	92.29±0.03[a]
50	42.32 ± 0.26	92.76 ±0.11[a]
75	64.30 ± 0.34[a]	93.22±0.03[a]
100	74.80 ± 0.23[a]	93.75 ±0.09[a]

[a]=Significant

When methanol extract of the investigated plant was tested for DPPH radical scavenging activity, it was found that 75µg/ml and 100 µg/ml of the extract lowered the DPPH radical levels above 64 and 74%, respectively. Inhibition of DPPH radicals above 50% is considered as significant for antioxidant properties of any compounds[17]. The scavenging activity of the extract used in the present study reflects the antioxidant properties but concentrations, which brought this change, are high as compared to standard reference used. This might be due to the crude methanol extract of the leaves of the investigated plant used in the study. This further suggests that scavenging activity of DPPH radical is present in few compounds of the extract used and there is need to purify and characterize the individual components and test them for their antioxidant properties both *in vitro* and *in vivo.*

Conclusion

The results obtained in the present study are in agreement to a certain degree with the traditional uses of the plant. The results of the screening assays confirmed the use of the investigated plants in traditional medicine of North East India and could form a good basis for selection of plant species for further investigation potential natural bioactive compounds. It is the first report about antioxidant effects of *D. sinuata* and its antibacterial activity against Gram-negative bacteria.

Acknowledgement

Authors are thankful to the village headmen and local medicine men, cooperated in carrying out the fieldwork. We are also thankful to Prof. S. K. Borthakur and Dr. G. C. Sarma, Department of Botany, Gauhati University for their technical guidance for this work.

References

1 World Health Organization (WHO) Report on Traditional Medicine Strategy 2002-2005, Geneva, 2002.

2 Dutta PK and Dutta BK, Medico-ethnobotanical studies on some North East Tribes/Ethnic community settled in Barak Valley, Assam, *In*: Environmental Issues and Priorities-Challenges of the Millennium (UGC sponsored National Seminar), 2000, pp. 6-7.

3 Dutta BK and Dutta PK, Potential of ethnobotanical studies in North East India: An overview, *Indian J Trad Knowledge,* 2005, **4**, 7-14.

4 Austin DJ, Kristinsson KG and Anderson RM, The relationship between the volumes of anti-microbial consumption in human communities and the frequency of resistance, *Proc Natl Acad Sci,* 1999, **96**, 115-1156.

5 Motsei ML, Lindsey KL, Van Staden J and Jaeger AK, Screening of traditionally used South African plants for antifungal activity against *Candida albicans*, *J Ethnopharmacol*, 2003, **86**, 235-241.

6 Samy RP, Ignacimuthu S and Sen A, Screening of 34 Indian medicinal plants for antibacterial properties, *J Ethnopharmacol,* 1998, **62**, 173-181.

7 Alma MH, Mavi A, Yildirim A, Digrak M and Hirata T, Screening chemical composition and *in vitro* antioxidant and antimicrobial activities of the essential oils from *Origanum syriacum* growing in Turkey, *Biol Pharm Bull*, 2003, **26**, 1725-1729.

8 Joyeux M, Moitier F and Fleurentin J, Screening of antiradical, antilipoperoxidant and hepatoprotective effects of nine plant extracts used in Caribbean folk medicine, *Phytotherapy Res*, 1995, **9**, 228-230.

9 Willcox JK, Ash SL and Catignani GL, Antioxidants and prevention of chronic disease, *Crit Rev Food Sci Nutr*, 2004, **44**, 275-295.

10 Ramteke A, Hasnu S, Borah S and Das RK, *In vitro* study of DPPH radical-scavenging activity of leaf extracts of *Phlogacanthus tubiflorus* Nees, *J Appl Biosci Biotechnol,* 2007, **3**(1), 74-77.

11 Jain SK and Rao RR, A Hand Book of Field and Herbarium Methods, Todays & Tomorrows Publication, New Delhi, 1977, pp.42-67.

12 Rojas A, Hernandez L, Pereda-Miranda R and Mata R, Screening for antimicrobial activity of crude drug extracts and pure natural products from Mexican medicinal plants, *J Ethnopharmacol,* 1992, **35**, 275-283.

13 Brand WW, Cuvelier HE and Berset C, Use of a free radical method to evaluate antioxidant activity, *Food Sci Technol*, 1995, **82**, 25-30.

14 Wagner H and Bladt S, Plants Drug Analysis: A Thin Layer Chromatography Atlas, Berlin-Springer, 1996, pp. 306-364.

15 Borthakur SK, Studies in ethnobotany of the *Karbis (Mikirs)*; Plant masticatories and dyestuffs, *In*: Contributions to Ethnobotany of India, edited by SK Jain, Scientific Publisher, Jodhpur, 1990, pp. 277-283.

16 Rawat MS and Choudhury S, Ethnomedicobotany of Arunachal Pradesh (Nishi and Apatani tribes), Bishen Singh Mahendra Pal Singh Publication, New Delhi, 1998, pp.123-129.

17 Sanchez-Moreno C, Larrauri JA and Saura-Calixto F, A procedure to measure the antiradical efficiency of polyphenol, *J Sci Food Agri*, 1998, **76**, 270-276.

18 Bauer AW, Kirby WMM, Sheriss JC and Turck M, Antibiotic susceptibility testing by standardized single method, *American J Clin Pathol,* 1996, **45**, 493-496.

19 Zhang X, Traditional medicine: its importance and protection. *In*: Protecting and Promoting Traditional Knowledge: Systems, National Experiences and International Dimensions, S Twarog and P Kapoor (Editors), New York, 2004, pp. 3-6.