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Research Article

DOCUMENTATION OF ETHNO-MEDICINAL PLANTS OF NADIA DISTRICT OF WEST BENGAL AND *IN VITRO* SCREENING OF THREE LOCAL MEDICINAL PLANTS FOR THEIR ANTIBACTERIAL ACTIVITY

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ABSTRACTS
Natural products especially plants have been used for the treatment of various diseases for thousands of years. The development of drug resistance as well as appearance of undesirable side effects of certain antibiotics has led to the search of new antibacterial agents in particular from medicinal plants. Higher plants have been shown to be a potential source for new anti-microbial agents. Contrary to the synthetic drugs, antimicrobials of the plant origin are not associated with many side effects and have an immense therapeutic potential to combat many infectious diseases. An attempt has been made to collect ethno-botanical information on medicinal plants being used in the district of Nadia of West Bengal, India. Direct discussion between the author and local healers and knowledgeable villagers were made and the uses of 67 plants were recorded. Among them, the crude leaf extract of *Clerodendrum viscosum, Centella asiatica* and *Nyctanthes arbortristis* were subjected to screen their antimicrobial activity against one gram positive bacteria and one gram negative bacteria. It is evident that the leaf extract of *Nyctanthes arbortristis* showed better antimicrobial activity compared to *Clerodendrum viscosum* and *Centella asiatica* against both Gram positive and negative bacterial strains.

Keywords: *Ethno-Medicinal Plants, Antimicrobial, Disc Diffusion Assay*

INTRODUCTION
Search for healing properties of plants to mitigate the misery of human beings caused by various ailments led to the study of plants around them and developed the science of support to life, that dates back to the days of Charaka Samhita and Sushruta Samhita (1,200 A.D) (Chopra *et al.,* 1956; Kirtikar and Basu, 1999; Rashed 2014). Addition of knowledge during passage of time helped to rebuild the modern medical system. The faster pace of life and the need of rapid cure facilitated the proliferation of synthetic drugs. The use of synthetic drugs raised several questions and that necessitates the use of natural products, emphasizing the development of herbal medicine. Since the world population is growing at an alarming rate, it increases the demand for necessary items, including medicines. Only 1% of known medicinal plant species (used as folk, ethno and traditional medicines) has so far been acknowledged, which encouraged WHO to promote and facilitate the study and practice of herbal medicine effectively in the developing countries as part of health-care programmes (Dhar *et al.,* 2002; Banerjee 2012). The Biological Diversity Act 2002 implies that all organisms are potent resources of considerable economic and social value, worthy of scientific investigation and of securing rights over associated intellectual property.

Modern medical systems have discovered many new antimicrobial compounds from various kinds of sources. Folk medicine is one such resource. Systematic study of them may result in the discovery of novel effective compounds (Tomoko *et al.,* 2002). The development of drug resistance as well as appearance of undesirable side effects of certain antibiotics (WHO, 2002; Bandow *et al.,* 2003) has led to the search of new antibacterial agents in particular from medicinal plants. Higher plants have been shown to be a potential source for new anti-microbial agents (Mitscher *et al.,* 1987; Jadhav *et al.,* 2013). The use of synthetic drugs raised several questions. Contrary to the synthetic drugs, antimicrobials of the plant origin are not associated with many side effects and have an immense therapeutic potential to combat many infectious diseases (Iwu *et al.,* 1999). According to W.H.O. medicinal plants would be the best source to obtain a variety of drugs (Santos *et al.,* 1995).

In view of this, an attempt has been made to collect ethno-botanical information on medicinal plants used in the district of Nadia of West Bengal, India and to focus on the *in vitro* antibacterial activity of three widely used plants which is relevant in the field of pharmacognosy and pharmacology. This ultimately may give an impetus to the pharmaceutical industry and thus economic development.

MATERIALS AND METHODS

Ethno-medicinal data were collected by consulting the local healers and knowledgeable villagers. The study was conducted in year 2011-2012 in Nadia district of West Bengal. A standard questionnaire was used to collect data, which includes local name of plants, plant parts used, methods of preparation and approximate dosage of administration. The uses of plant species were verified in other villages cross checking information with other respondents showing plant species in natural habitat or collected sample. The data were considered valid if at least five informants provided similar uses about medicinal plants.

Photograph of each specimen has been taken by digital camera, carefully collected, labeled, numbered, and annotated with date and place of collection. Identification of the specimen has been done after critical examination and comparison with the authentic materials available in the Herbarium with the help of the Taxonomists of the Department of Botany, Krishnagar Govt. College. Voucher specimens have been deposited at the Herbarium of the Department of Botany, Krishnagar Govt. College.

Assessment of Antimicrobial Activity

Preparation of Crude Leaf Extract: Leaves of *Clerodendron viscosum, Centella asiatica* and *Nyctanthes arbor-tristis* were dried in shade and stored in cotton bags and then finely powdered (100 g) separately with the help of a grinder. Each ground material was soaked in 500 ml ethyl alcohol for 72 hour and filtered. The filtrate was then allowed to evaporate in rotary evaporator until completely dry and kept in a refrigerator for further study. Then the dried extract, was weighed and dissolved in sterile distilled water. The concentrations of the final extracts were made as 50 mg/1ml.

Assay of antimicrobial activity: Antimicrobial activity was determined as diameter of inhibition zone using disc diffusion method (Bauer *et al.*, 1966). Inhibition zone of all extracts were compared to the standard antibiotic (Ciprofloxacin 10 µg/disc). Nutrient agar (NA) was distributed in sterilized Petri dishes for the preparation of bacterial lawn. One gram positive bacterial strains, *Bacillus subtilis* and one gram negative bacterial strains, *Escherichia coli* were employed in this test. These species were obtained from the mother stock of the Microbiology Laboratory, Department of Botany, Visva-Bharati, Santiniketan. Small paper discs of 6 mm in diameter were soaked with 10 µl (500 µg) of each leaf extract. Those discs were placed on agar growth medium containing a confluent lawn of microorganism. Minimum Inhibitory Concentrations (MIC) was determined by serial dilution technique.

Culture media and inoculums: Solid Nutrient agar media was prepared. About 25 ml of media was poured into a Petri dish. The inoculums were prepared by culturing the organisms in a test tube containing 10 ml liquid media for bacterial strains and incubating over night at 37^{o}C. The agar plates for the assay were prepared by labeling them with the date, the name of the microorganism and the name (code) of the discs. The inoculums of bacteria were transferred into Petri dish containing solid nutrient media of agar using a sterile swab. The swab was used to spread the bacteria on the media in a confluent lawn. One swab was used for one species of bacteria.

Placing test discs: Dried test discs were transferred on bacterial lawn under aseptic conditions using spirit-flame sterilized forceps each time. Each disc was placed gently on the agar surface and plated with the forceps so that it sticks. Resulting zones of inhibition were observed and measured in millimeters. Tests were repeated in triplicate and were performed to insure reliability of the results.

RESULTS AND DISCUSSION

Direct discussion between the author and local healers and knowledgeable villagers of the district Nadia of West Bengal (Figure 1) were made and the uses of the plants were recorded, mentioned in Table1.

Plants are always surrounded by an enormous number of potential enemies such as bacteria, viruses, fungi, insect etc. (Van Wyk & Gericke, 2000). It is logical to expect biologically active compounds to be

produced by plants as a chemical defense measure against their enemies. Natural products have been a consistently successful source in drug discovery and offer more opportunities to find antimicrobial drugs or lead compounds (Wang *et al.,* 2006). Plants synthesize very complex molecules with specific stereochemistry and can show biological activity with novel modes of action (Houghton, 1996).

However, all the crude extracts of leaves of *Clerodendrum viscosum, Centella asiatica* and *Nyctanthes arbortristis* were subjected to screening against one gram positive bacteria and one gram negative bacteria. It is clear from inhibition zones (Table 2) that all the leaf extracts were effective against both gram positive and gram negative bacteria. It is evident from the available data that the leaf extract of *Nyctanthes arbortristis* showed better antimicrobial activity compared to *Clerodendrum viscosum* and *Centella asiatica* against these two bacterial strains (Figure 2).

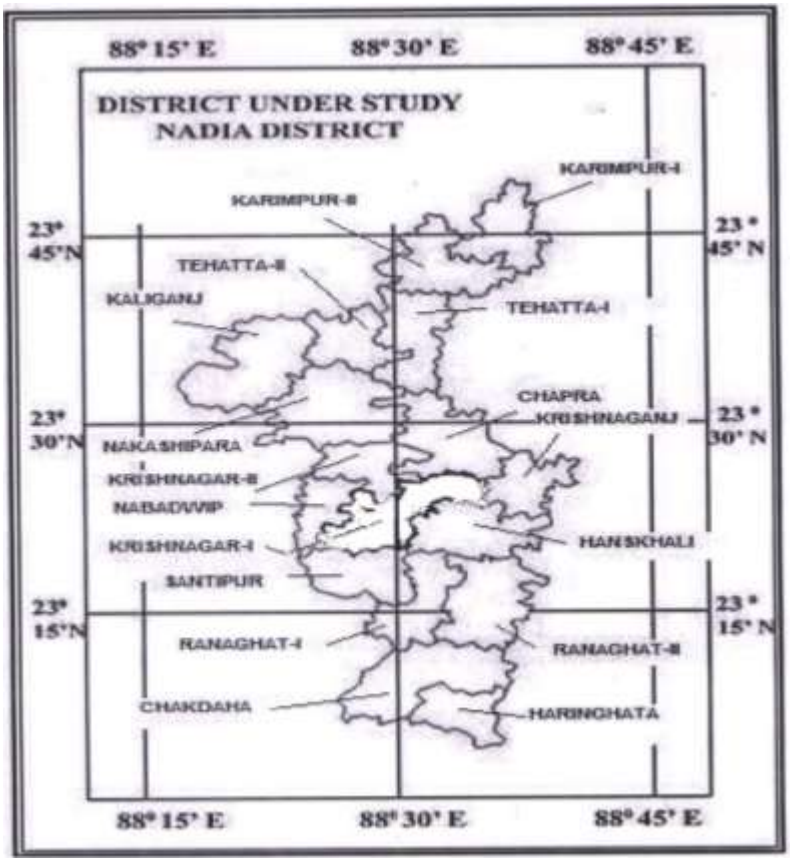

Figure 1: Map of Nadia District

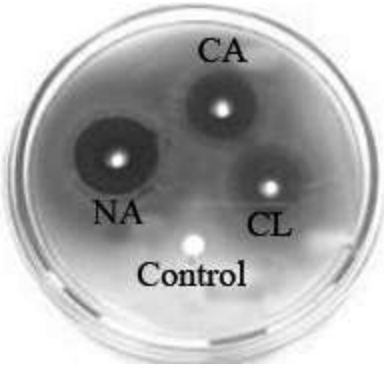

Figure 2: Antimicrobial activity of leaf extracts of Centella asiatica (CA), Nyctanthes arbor-tristis (NA) and Clerodendrum viscosum (CL); Zone of inhibition formed on disc diffusion test

Table1: Documentation of some commonly used Ethno-Medicinal plants in the district of Nadia of West Bengal

No.	Botanical Name	Local Name	Family	Parts Used	Uses
1.	*Achyranthes aspera* Linn.	Apang	Amaranthaceae	Whole plant	Diuretic, Cardiac stimulant
2.	*Acorus calamus* Linn.	Bach	Araceae	Rhizome	Stomachic, anti-flatulent, cough, bronchitis
3.	*Adhatoda vascica* Nees	Vasaka	Acanthaceae	Leaves, flowers, fruits, roots	Expectorant
4.	*Aegle marmelos* (Linn.) Corr.ex Roxb	Bael	Rutaceae	Leaves, fruits	Antidiabetic, laxative
5.	*Allium cepa* Linn.	Peyaj	Liliaceae	Bulbs	Conjunctivitis, insect bite
6.	*Allium sativum* Linn.	Rasun	Liliaceae	Bulbs	Antirheumatic, Anti-inflammatory, hypo-cholesterolemic, hypotensive, antidiabetic
7.	*Andrographisp paniculata* (Burm.f.) Wall ex. Nees	Kalmegh	Acanthaceae	Whole plant	Antipyretic, skin diseases, liver complaints
8.	*Asparagus racemosus* Willd.	Shatamuli	Liliaceae	Root, leaves	Hyper-acidity, peptic ulcer, aphrodisiac
9.	*Azadirachta indica* A. Juss	Neem	Meliaceae	Leaves, Bark, seeds, oil	Antiviral, Pox, eczema and skin diseases
10.	*Bacopa monnieri* (Linn.)	Brahmi	Scrophulariaceae	Leaves	Improvement of intelligence and memory and revitalization of sense organs
11.	*Bauhinia variegata* Linn.	Kanchan	Caesalpiniaceae	Roots, leaves, bark, seeds	Dysentery, diarrhoea, piles, worms, antidote to poison
12.	*Boerhaavia diffusa* Linn.		Nyctaginaceae	Whole plant	Laxative
13.	*Butea monosperma* (Lam.) Taub.	Palash	Papilionaceae,	Bark, leaves, seeds, flower	Anthelmintic, Oral contraceptive
14.	*Calotropis gigantea* (Linn.) R. Br.	Akanda	Asclepiadaceae	Whole plant	Aanthelmintic, expectorant
15.	*Carica papaya* Linn.	Pepe	Caricaceae	Fruits, latex	Oral contraceptive, anti-fertility, digestant, anthelmintic
16.	*Catharanthus roseus* (Linn.) G.Don.	Nayantara	Apocynaceae	Roots, leaves	Antidiabetic
17.	*Centella asiatica* (Linn.)	Thankuni	Apiaceae,	Whole plant	Antidysenteric, brain tonic
18.	*Cinnamomum camphora* Nees & Eberm.	Karpoor	Lauraceae,	Oil	Carminative
19.	*Cleome viscosa* Linn.	Hulhuria	Capparidaceae	Whole plant	Antifungal, anthelmintic, carminative
20.	*Clerodendrum viscosum* Vent.	Ghentu	Verbenaceae	Leaves	Antiinflammatory, vermifuge
21.	*Coriandrum sativum* Linn.	Dhone	Apiaceae	Fruit	Carminative, antibilious
22.	*Costas speciosus* (Koen.) Sm.	Keu	Zingiberaceae	Rhizomes	Oral contraceptive
23.	*Curcuma amada* Roxb.	*Amada*	Zingiberaceae	Rhizomes	Sprains, gout, inflammations
24.	*Curcuma longa* Linn.	Halud	Zingiberaceae	Rhizomes	Ringworm, itching, eczema, chicken pox, cold, cough, bronchitis, liver troubles, antidysenteric, sprains and bruises

25. *Curcuma zedoaria* (Berg.) Rosc.	Sati	Zingiberaceae	Rhizomes	Appetiser and tonic, particularly prescribed to ladies after childbirth
26. *Cynodon dactylon* (Linn.) Pers.	Durba Ghas	Poaceae	Whole plant	Haemostat
27. *Datura metel* Linn.	Dhutura	Solanaceae	Whole plant	Emetic, narcotic, antispasmodic antiasthamatic, anti inflammatory
28. *Eclipta alba* Hassk.	Kesutthe	Asteraceae	Whole plant	Hepatitis, spleen enlargements, an antidote in scorpion sting
29. *Emblica officinalis* Gaertn.	Amlaki	Euphorbiaceae	Fruits, leaves, tender shoots, seeds	Carminative, stomachic, antianaemic, bronchitis, anti inflammatory, dyspepsia, dysentery
30. *Euphorbia hirta* Linn.,	Dhudhi	Euphorbiaceae	Leaves	Diuretic, aphrodisiac
31. *Gloriosa superba* Linn.	Bishalanguli	Liliaceae	Roots, rhizomes	Abortifacient, antipyretic, anti inflammatory, antidote against cobra poison, poisonous insects, snake bites, scorpion sting
32. *Glycosmis pentaphylla* (Retz.) DC.	Ashshoura	Rutaceae,	Whole plant	Antiinflammatory, antianaemic, antirheumatic
33. *Gymnema sylvestris* (Retz.)R. Br.	*Gurmar*	Asclepiadaceae	Leaves, Roots	Anti-diabetic, snakebite
34. *Hibiscus rosa sinensis* Linn.	Jaba	Malvaceae	Roots, leaves, Flower,	Febrifuge, emollient, demulcent
35. *Holarrhena pubescens* (Buch.-Ham.) Don	kurchi	Apocynaceae,	Bark, leaves,	Antidysenteric , carminative, expectorant
36. *Hygrophila schulli* (Ham.) M. R. & S. M. Almeida	Kule khara	Acanthaceae	Roots, leaves, seeds	Antianaemic, aphrodisiac
37. *Lantana camara* Linn. var. *aculeata* Moldenke	Putus	Verbenaceae	Whole plant	Antirheumatic
38. *Mimosa pudica* Linn.	Lajjwabati	Mimosaceae	Roots, leaves	Carminative, aphrodisiac
39. *Mimusops elengi* Linn.	Bakul	Sapotaceae	Unripe fruit	masticatory to fix loose teeth
40. *Momordica charantia* Linn.	Karala	Cucurbitaceae	Fruits	Antidiabetic
41. *Moringa oleifera* Lam.	Sajna	Moringaceae	Root, bark, leaves, seed,	Abortifacient
42. *Murraya koenigii* (Linn.) Spreng.	Curry pata	Rutaceae	Bark, root, leaves	Febrifuge, stomachic
43. *Nyctanthes arbor-tristis* Linn.	Shiuli	Oleaceae,	Leaves	Febrifuge, antiinflammatory
44. *Ocimum tenuiflorum* Linn.	Tulsi,	Lamiaceae	Leaves, root, seed	Expectorant, ring worm
45. *Phyllanthus amarus* Schum. & Thonn.	Bhui amla	Euphorbiaceae	Whole plant	Antihepatotoxic
46. *Piper longum* Linn.	Piplamul	Piperaceae	Root, fruit, dried spikes	Bronchitis, cough
47. *Psidium guajava* Linn.	Peyara	Myrtaceae	Leaves	Haemostatic, antiemetic, dentalgia
48. *Raphanus sativus* Linn.	Mula	Brassicaceae,	Root, leaves	Appetising, antiflatulent, digestive
49. *Rauvolfia serpentina* Benth. ex Kurz..	Sarpagandha	Apocynaceae	Root	Antihypertensive

50.	*Ricinus communis* Linn.	Bherenda	Euphorbiaceae	Leaves, oil	Laxative, rheumatoid arthritis
51.	*Santalum album* Linn.	*Swetchandan*	Santalaceae,	Heart wood	Pimples,
52.	*Saraca asoca* (Roxb.) de Wilde.	Ashok.	Caesalpiniaceae	Bark, Leaves	Dyspepsia, fever, menorrhagia, leucorrhoea, pimples
53.	*Sida cordifolia* Linn.	Berela	Malvaceae	Root, seed	Aphrodisiac
54.	*Solanum nigrun* Linn.	Kakmachi	Solanaceae	Whole plant	Swellings, flatulence, dyspepsia, splenomegaly,
55.	*Solanum torvum* Sw.	Titbaigan.	Solanaceae	Fruits	Spasmolytic
56.	*Solanum xanthocarpum* schrad. & Wendl.	Kantakari	Solanaceae	whole plant	Dental caries, fever, cough, asthma, bronchitis
57.	*Syzygium cumini* (Linn.) Skeels,	Jam	Myrtaceae	Seed powder leaves	Antidiabetic, carminative
58.	*Tabernaemontana divaricata* (Linn.) Roem. & Schult.	Kolke	Apocynaceae	Latex, flower	Antiinflammatory, antidiarhhoeal
59.	*Terminalia arjuna* (Roxb.ex DC) Wight & Arn.	Arjun	Combretaceae	Bark	Cirrhosis of lever, leucorrhoea, diabetes, anaemia, hypertension
60.	*Terminalia belerica* (Craertn.) Roxb.	Bahera	Combretaceae	Fruit, bark	Bile-stimulant, stomachic, anaemia, leucoderma, bronchitis
61.	*Terminalia chebula* Retz.	Haritaki	Combretaceae	Fruit, bark	Dental caries, cardiac tonic
62.	*Tinospora cordifolia* (Willd.) Hook. f. & Thom.	Gulancha	Menispermaceae	Stem, leaves, root	Sexual impotency, hypoglycaemic
63.	*Trigonella foenumgraecum* Linn.	Methi	Papilionaceae,	Leaves, seed	Antidiabetic, hypotensive
64.	*Tylophora indica* (Burm. f.) Merr.	Antamul,	Asclepiadaceae,	Root, leaves,	Emetic, cathartic
65.	*Vitex negundo* Linn.	Nishindha	Verbenaceae	Leaves ,	Anti-rheumatic, Anti-inflammatory
66.	*Withania somnifera* (Linn.) Dunal	Ashwa gandha	Solanaceae	Root, leaves	Rejuvenating, sound sleep, joint pains, inflammation, Sexual impotency
67.	*Zingiber officinale* Rosc.	Ada	Zingiberaceae,	Rhizome	Stomachic, cough and cold

Table 2: Assay of antimicrobial activity of leaf extracts of *Clerodendrum viscosum, Centella asiatica* and *Nyctanthes arbortristis* using disc diffusion method

Name of the organism	Diameter of the zone of inhibition (in mm) including diameter of the disc (6mm) and MIC							
	Clerodendrum viscosum (500 µg/disc)		*Centella asiatica* (500 µg/disc)		*Nyctanthes arbor-tristis* (500 µg/disc)		Ciprofloxacin (10 µg/disc)	
	Zone of inhibition (in mm)	MIC (µg/ml)	Zone of inhibition (in mm)	MIC (µg/ml)	Zone of inhibition (in mm)	MIC (µg/ml)	Zone of inhibition (in mm)	MIC (µg/ml)
Bacillus subtilis	11	100	11	100	16	50	22	26
Escherichia coli	9	150	10	150	18	50	24	20

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