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Ethnomedicinal plants of Assam, India as an Alternative source of future Medicine for Treatment of Pneumonia

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Abstract

In Assam there are several plants used in folk medicine for treatment of pneumonia but for integration of these ethnomedicine into modern medicine system there must be scientific proof for the bioactivity of these traditionally used plants. The present work was conducted to asses antibacterial activity of selected ethnomedicinal plant species used traditionally for pneumonia treatment in Assam. Eighty plant extracts (prepared with solvents with increasing polarity) from 20 plants belonging to 17 families were screened for their antibacterial activity using disc diffusion method against two Gram positive (Staphylococcus aureus [MTCC96] , Streptococcus pneumoniae [MTCC655]) and two Gram negative (E.coli [MTCC443] and Pseudomonas aeruginosa [MTCC424]) bacteria. Out of the twenty plants screened fourteen plants showed varying degree of antibacterial activity against at least one human pathogen tested. Chloroform extract of Mucuna pruriens and petroleum ether extract of Xanthium strumanium showed strong antibacterial activity with higher zone of inhibition than the control viz. chloramphenicol against S. pneumoniae (15.33mm) and S. aureus (15mm) respectively. The present study provides scientific evidence for the use of majority of the studied medicinal plants including Mucuna pruriens and Xanthium strumanium in traditional medicine and these could serve a potential source of alternative antibacterial drugs in future.

Keywords: Medicinal plants, ethnomedicinal knowledge, antibacterial activity, pathogenic bacteria, pneumonia.

Introduction

Pneumonia is an infectious disease which is a common cause of morbidity and mortality worldwide[1]. Pneumonia is one of the leading causes of child mortality in India. Treatment of pneumonia become challenging specially in developing countries due to rapid development of antibiotic resistant bacteria[2-5]. Although various antimicrobial agents have been newly introduced, microbes develop resistant to these drugs so rapidly that the drugs have a short life expectancy[6]. Researchers are trying to develop better and less expensive drugs from herbal products against multidrug resistant microbial strains because plants have an inherent capacity to fight against pathogens by developing secondary metabolites. Besides microbes are less likely to develop resistance against plant medicines. Currently plant derived pharmacologically active compound are considered to be a useful source of antimicrobial drug for resistant microbes[7]. From ancient time plant is used as a source of antimicrobial agent due to their easy availability and less side effects[8]. But to integrate plant medicines in health care there must be scientific validation for their medicinal activity.

According to the World Health Organization (WHO) 65% of the world population rely on ethnomedicinal plants to meet their primary health care needs[9]. WHO has reported that sales of herbal medicines is increasing globally at a rate of 7-10% every year and is likely to increase from around US $ 120 billion to more than US $ 5 trillion in next 35 years[10-11]. In India various ethnic groups have been using crude extracts of different plant parts such as leaves, fruit, roots or bark in the form of decoctions, infusions or concoctions for treatment of infectious diseases from remote past[12-13]. In recent years the antimicrobial drugs developed are mostly bacterial or fungal in origin that has a limited effective life span[8]. Therefore microbiologists are showing significant interest in finding new antimicrobial agents from medicinal plants.

In the North Eastern region of India including Assam, several workers have significantly reported various ethnomedicinal information[14]. But to our knowledge there is no published report on antibacterial activity of herbal medicines used for treatment of pneumonia by the local people or traditional healers in Assam. Assam is rich in medicinal flora and traditional folk medicinal practices that can be exploited to develop novel drugs. The present study was conducted to determine antibacterial activity of various ethnomedicinal plants used by the traditional herbal healers and the local people of Assam to treat pneumonia.

Material and Methods

Preparation of plant extract: Plants were collected on the basis of ethnomedicinal survey in its flowering state as far as possible in the field following standard methods of plant collection[15]. Plant parts were shade dried and ground to fine powder by using an electric grinder. Ground samples were

extracted with a series of solvents, petroleum ether, chloroform, methanol in sequence of increasing polarity at room temperature using soxhlet apparatus and concentrated extracts were obtained using rotary vacuum evaporator[16]. Water extract was prepared by pouring double distilled water onto the fine powder and kept for 72 h at room temperature. The water is then filtered after refluxing over hot water bath and then again water is added. This process was repeated for several times and the filtrate was then evaporated to dryness using rotary vacuum evaporator[16].

Microbial strains: Four human pathogenic reference bacteria were used in this study, two gram positive [*Staphylococcus aureus* (MTCC 96) and *Streotococcus pneumoniae*(MTCC 655)] and two gram negative [*E. coli* (MTCC 443) and *Pseudomonas aeruginosa* (MTCC 424)] obtained from collection of Microbial Type Culture Collection and Gene Bank (MTCC). The bacterial cultures were maintained at 4 °C on nutrient agar.

Antibacterial Assay: Antibacterial study was carried out by disc diffusion method[17] against the pathogenic strains. Standardized inoculums (1×10^5 CFU/ml, 0.5 McFarland standards) were spread evenly with a glass spreader over the surface of the plate containing Muller Hinton Agar media (MHA). The 6mm diameter discs (Whatman filter paper No. 1) loaded with plant sample (1mg/disk) were placed on the surface of the medium and left for 30 min at room temperature under laminar flow for compound diffusion. Chloramphenecol (10 µg per disc) and blank discs with DMSO were used as positive control and negative control respectively. The plates were incubated for 18 h at 37^0. Each sample was tested in triplicate and zone of inhibition was recorded in millimeters.

Results and Discussion

Table 1 provides the botanical name, family, local name, and plant parts used together with their traditional therapeutic uses collected from different parts of Assam. The results of the Disc Diffusion of the plants extracts showing bioactivity were depicted in table 2. The results obtained in the present study revealed that out of 20 plants and 80 plant extracts tested, 14 plants and 45 extracts exhibited potential antibacterial activity against the tested bacterial pathogens with varying zone of inhibition. The petroleum ether extract of all the 14 plants showed antibacterial activity against one or more pathogens. The petroleum ether extract of *M. pruriens*, *C. viscosum, Oroxylum indicum* and *Solanum indicum* showed inhibitory effect against all the four strains. The chloroform extract of *M. pruriens* and *Polygonum hydropiper* was also effective against all the tested pathogens. Chloroform and petroleum ether extracts of these plants showed activity towards Gram positive and Gram negative bacteria probably due to the presence of broad spectrum antibacterial compounds. Similar results also reported in case of petroleum ether and chloroform extract of *Nerium oleander* leaves[18]. Results of the present work showed that extracts of few plants could serve as potent source of antibacterial agents as these extracts showed inhibitory effect more than or equal to that of positive control such as petroleum ether extract of *Xanthium strumanium* and chloroform extract of *Mucuna pruriens* showed higher degree of zone of inhibition than that of positive control against *Staphylococcus aureus* (15 mm) and *Streptococcus pneumoniae* (15.33 mm) respectively. Furthermore, petroleum ether extract *P. thyrisiflorus* and chloroform extract of *L. aspera* showed inhibition zone almost equal to that of positive control against *E. coli.* Chloroform extract of 12 plants showed antibacterial activity. Chloroform extract of *Musa balbisiana, Solanum indicum and Nyctanthus arbor- tritis* showed antibacterial activity against Gram negative bacteria viz. *E. coli* and *P. aeruginosa*. Chloroform extract of *C. asiaticum, P. thyrsiformis and P. longum* were found effective against both the Gram positive strains *viz S. aureus and S. pneumoniae* whereas *X. strumanium* showed inhibitory effect only against *S. aureus.* Chloroform extract of *Clerodendrum viscosum, Leucas aspera* and *Oroxylum indicum* showed zone of inhibition against three out of four pathogens viz. *P. aeruginosa*, *S. aureus, and S. pneumonia; E.coli, P. aeruginosa and S. aureus; E.coli, P. aeruginosa and S. pneumonia* respectively. Methanol extract of *C. viscosum* was effective against all the four pathogens tested and that of *L. aspara* was inhibitory to three pathogens, E.*coli, P. aeruginosa* and *S. aureus*. Methanol extract of *M. pruriens* and *S. indicum* showed bioactivity against one gram positive (*S. pneumonia*) and one gram negative (*P. aeruginosa*) strain where as zone of inhibition for *P. hydropiper* methanol extract was observed against both the gram positive strains tested and that of *P. thyrisiformis*, *M. balbisiana* were active against only *S. aureus*. *S. media* and *C. asiaticum* methanol extracts were effective only against *E. coli*. Zone of inhibition was also observed for *X. strumanium* methanol extract against *P. aeruginosa* and *S. pneumonia* All the four bacteria were sensitive to the plant extracts, but *S. aureus* was found to be most sensitive, which was inhibited by petroleum ether extract of 14 plants, chloroform extract of 8 plants, methanol extract of 6 plants and aqueous extract of 7 plants. Similar results observed by previous scientists with extracts of *Callistemon lanceolatus* against uropathogenic bacteria[19]. Gram positive bacteria were more sensitive towards the plant extracts than the Gram negative bacteria. Similar work was also reported earlier[20-22].

Assam, a state of North East India is one of the biodiversity hotspots and a rich reservoir of plant diversity that harbours many rare and endemic plants. There are several ethnic communities in Assam including 5 major tribes and 9 minor tribes[23]. The ethnic groups have a rich source of traditional knowledge system and are known to use ethnic traditions for curing various primary healthcare problems[24]. Majority of the tribal communities in Assam in rural and hilly areas are mainly dependant on herbal medicines for treatment of some common diseases such as cold, cough, asthma, headache, fever, pneumonia, skin diseases, stomachache and many others. Ethnomedicinal knowledge of various ethnic and non- ethnic communities of Assam could lead to the development of new

and better drugs for treatment of infectious diseases. Therefore, there is an urgent need to preserve and validate scientifically this traditional knowledge as the new generation shows least interest in continuing the indigenous knowledge system of their forefathers. Besides the destruction of forest, urbanization and industrialization posing major threat to various plant species in Assam[23].

The search for novel antimicrobial compounds is a never ending process because microbes have tendency to develop antibiotic resistance. There is a need for further investigation of the bioactive crude extracts specially petroleum ether extract of *Xanthium strumanium* and chloroform extract of *Mucuna pruriens* to develop new antibacterial agents that may be useful in treatment of various infectious diseases.

Table-1
List of medicinal plants used for treatment of pneumonia in varrious parts of assam

Plant species	Family	Vernacular name	Parts used	Medicinal uses
Acorus Calamus L.	Araceae	Bach	Leaves, rhizome	Cough, bronchitis, pneumonia
Crinum asiaticum L.	Amaryllidaceae	Bon nohoru	Bulb	Fever, headache and cough, pneumonia
Cymbopogon flexuosus (Steud.) Wats	Poaceae	Lemongrass	Leaves	Respiratory infections as sore throats and fever, pneumonia
Lantana Camara L.	Verbenaceae	Gui ful	Leaves	Fever, asthma, pneumonia
Solanum Indicum Linn.	Solanaceae	Tit bhekuri	Fruits, leaves	Bronchitis, asthma, pneumonia,cough
Solanum torvum Sw.	Solanaceae	Hativekuri	roots	Cough, headache,pneumonia
Alstoia scholaris (L) R. Br.	Apocynaceae	Satiyana	bark	Fever, bronchitis, pneumonia
Clerodendrum viscosum Vent.	Verbenaceae	Bhetei tita	roots	Bronchitis and pneumonia
Stellaria media L	Caryophyllaceae	Moreleia	leaves	Pneumonia
Leucas aspara (Wild.) Link.	Labiatae	Duron	Leaves, flower	Cough, fever,pneumonia
Mucuna pruriens DC.	Fabaceae	Bandar Kekua	seeds	Bronchitis, pneumonia
Mirabilis jalapa L.	Nyctaginaceae	Godhuli Gopal	Rhizome, leaves	Fever, headach, pneumonia
Musa balbisiana Coll.	Musaceae	Athiya kol	rhizome	Pneumonia
Nyctanthes arbor –tristis L.	*Oleaceae*	Sewali	leaves	Fever, respiratory infections ,pneumonia
Oroxylum Indicum (L.) Vent.	Bignoniaceae	Bhatghila	bark	Cough and high fever, pneumonia
Phlogacanthus thyrsiformis (Hardw.) Mabb.	Acanthaceae	Tita phul	flower	Pneumonia
Piper longum L.	Piperaceae	Pippali	Leaves	Pneumonia, cough and respiratory diseases
Xanthium strumanium L.	Asteraceae	Agara	seeds	Pneumonia
Polygonum hydropiper L.	Polygonaceae	Bihlongoni	leaves	Headache, fever, pneumonia
Polygonum caespitosum Blume	Polygonaceae	Modhushulen	leaves	Pneumonia

Table-2
Antibacterial activity of the collected plants using disc diffusion method

Plant name	Pathogen	Diameter of the inhibition zone (mm) ***					
		Petroleum ether	Chloroform	Methanol	Water	Positive control*	Negative control**
Mucuna pruriens DC.	*E.coli*	7.5±0.5	10.33±0.58	-	-	20.68±0.58	-
	Pseudomonas aeruginosa	7.3±0.58	7±0	7.68±0.58	7±0.5	-	-
	Staphylococcus aureus	11.68±0.58	8.33±1.15	-	-	16.68±0.58	-
	Streptococcus pneumoniae	8±0	15.33±1.15	7±0	-	14.33±2.08	-
. *Polygonum caespitosum* Blume	*E.coli*	6.67±0.58	-	-	-	20.5±0.5	-
	Pseudomonas aeruginosa	7.5±0.5	-	-	-	-	-
	Staphylococcus aureus	7.67±0.58	-	-	8.17±0.29	19.67±0.58	-
	Streptococcus pneumoniae	-	-	-	-	18.67±0.58	-
Musa balbisiana Coll.	*E.coli*	-	8.33±1.15	-	-	20.83±0.28	-
	Pseudomonas aeruginosa	-	7.5±0.5	-	-	5.33±4.7	-
	Staphylococcus aureus	8.16±0.28	-	7.33±0.28	-	20.5±0.5	-
	Streptococcus pneumoniae	7.16±0.29	-	-	-	12.83±1.04	-
Xanthium strumanium L.	*E.coli*	-	-	-	-	16.67±2.89	-
	Pseudomonas aeruginosa	6.5±0.5	-	7.67±0.58	-	0	-
	Staphylococcus aureus	15±2.64	6.83±0.29	7±0	4.67±4.04	10.17±0.29	-
	Streptococcus pneumoniae	-	-	-	-	11.5±0.87	-
Solanum indicum Linn.	*E.coli*	7.33±0.29	-	-	-	9.33±1.154	-
	Pseudomonas aeruginosa	7.5±0.87	6.8±0.28	8.33±0.58	7.5±0.5	-	-
	Staphylococcus aureus	8±0	-	-	-	11.67±1.5	-
	Streptococcus pneumoniae	8.17±0.29	-	4.67±4.04	-	10.67±0.58	-
Clerodendrum viscosum Vent.	*E.coli*	6.83±0.29	-	7±0	7.3±0.58	20.67±0.58	-
	Pseudomonas aeruginosa	4.67±4.04	7±0	4.5±3.9	6.67±0.28	4.67±4.04	-
	Staphylococcus aureus	13.33±1.15	7.67±0.58	8.33±0.58	7±0	21±1.0	-
	Streptococcus pneumoniae	7±0	7±0	-	8.67±0.58	11.67±0.58	-
Phlogacanthus thyrsiformis (Hardw.) Mabb.	*E.coli*	11±1	-	-	-	12±1	-
	Pseudomonas aeruginosa	-	-	-	-	-	-
	Staphylococcus aureus	7.33±0.58	7±0	7.33±0.58	7±0	11±1	-
	Streptococcus pneumoniae	8±0	7.33±0.58	-	-	11±1	-

Leucas aspara (Wild.) Link.	*E.coli*	-	9.67±0.58	8±0	-	11.67±1.15	-
	Pseudomonas aeruginosa	-	7±0	7±0	-	-	-
	Staphylococcus aureus	7±0	7.67±0.58	7±0	-	10.33±1.52	-
	Streptococcus pneumoniae	-	-	-	-	17.67±0.58	-
Piper longum L.	*E.coli*	-	-	-	-	14±1.73	-
	Pseudomonas aeruginosa	-	-	-	-	-	-
	Staphylococcus aureus	8.5±0.5	7.33±0.5	-	-	20.33±0.58	-
	Streptococcus pneumoniae	8±0	7±0	-	-	15.33±1.15	-
Polygonum hydropiper L.	*E.coli*	-	7.67±0.58	-	-	9.5±0.87	-
	Pseudomonas aeruginosa	-	7.67±0.58	-	-	-	-
	Staphylococcus aureus	7.5±0.5	8.17±0.29	7.33±0.58	-	9.67±0.58	-
	Streptococcus pneumoniae	8±0	7.33±0.29	8.17±0.29	-	12.67±0.58	-
Crinum asiaticum L.	*E.coli*	-	-	-	-	10.17±0.29	-
	Pseudomonas aeruginosa	-	-	-	-	-	-
	Staphylococcus aureus	7.67±0.58	9.33±0.58	0	10.3±2.08	16±2.64	-
	Streptococcus pneumoniae	4.67±4.04	7.17±0.29	7.67±0.58	-	11±1	-
Oroxylum Indicum (L.) Vent.	*E.coli*	7.67±0.58	8	-	-	10.33±1.15	-
	Pseudomonas aeruginosa	7.8±0.29	7.17±0.29	-	-	-	-
	Staphylococcus aureus	7±0	-	-	4.67±4.04	16.33±2.08	-
	Streptococcus pneumoniae	8±1	8.33±0.29	-	-	11.67±1.52	-
Nyctanthes arbor –tristis L.	*E.coli*	-	7.17±0.29	-	-	18.67±1.15	-
	Pseudomonas aeruginosa	-	-	-	-	-	-
	Staphylococcus aureus	7.67±0.58	-	-	7±0	9.33±1.15	-
	Streptococcus pneumoniae	-	-	-	-	10.67±0.58	-
Stellaria media L	*E.coli*	-	-	7±0	-	20.67±0.58	-
	Pseudomonas aeruginosa	-	-	-	-	-	-
	Staphylococcus aureus	7±0	-	-	-	11±1.73	-
	Streptococcus pneumoniae	-	-	-	-	8.67±0.29	-

* represents Positive control (Chloramphenicol), ** representsNegative control DMSO (Dimethyl Sulfoxide), *** are Mean± Standard deviation (n=3 observations), ± represents standard errors of mean, - represents no zone of inhibition

Conclusion

From the results of the present work it is clear that seed extract of *Mucuna pruriens* and *Xanthium strumarium* is effective in controlling growth of both Gram positive and Gram negative strains. Besides petroleum ether extract *P. thyrisiflorus* and chloroform extract of *L. aspera* also showed inhibitory effect against *E.coli* almost equel to that of positive control. The present investigation revealed petroleum ether was the most effective crude extract followed by chloroform and methanol extract showing inhibitory effect against all the tested strains. Water extract was found to be least effective. The present work provides a scientific evidence of the traditional knowledge system of Assam as most of the ethnomedicinal plants collected showed a varying degree of antibacterial activities against the selected pneumonia causing pathogens and these plants may represent a safe alternative for treatment of pneumonia.

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