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Antibacterial activity of some ethno-botanically important ferns of Southern Assam, India

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ABSTRACT: Pteridophytes form an important part of the biodiversity of this blue green planet. It has been agreed by recent workers that pteridophytic population of India is about 10% of the total pteridophytic flora of the world. Besides showing economic values towards food and fodder, indicators, bio fertilizers, insect repellents etc. quiet a large number of pteridophytic species are used by different communities as medicine and folk medicines. They treat cuts and wounds and many other diseases with leaf extract/rhizome extract of pteridophytes. The tribal people of Barak Valley area of Southern Assam, are using different species of pteridophytes to cure various diseases.

In the present communication, total 21 species of common pteridophytes were enumerated from Barak Valley. The ethnobotanical importance of these species as reported by different authors were reviewed and presented in tabulated form. Further, frond extract of these species were tested against five potentially pathogenic microorganisms. The result showed that 10 species have antibacterial activity against one or more organisms while the remaining 11 species did not show any activity against any of the test organisms. The ferns showing antibacterial activity are *Adiantum capillus-veneris*, *Asplenium nidus*, *Cyathea brunoniana*, *Cyathea gigantea*, *Dipteris wallichii*, *Drynaria quercifolia*, *Lygodium japonicum*, *Lygodium flexuossum*, *Pityrograma calomelanos*, and *Pteris biaurita.*

KEY WORDS: Antibacterial screening, ethno-botany, Ferns, India, Southern Assam.

INTRODUCTION

The vast number of chemicals which are used in pharmaceutical industry are often hazardous to human health, it has been suggested that bioactive natural products are safe and useful as medicine. Moreover, the microorganisms have a tendency to become resistant to antimicrobial substances which are in use. Initially all strains of *Staphylococcus aureus* were sensitive to Penicillin, then they became resistant to penicillin but they were sensitive to methicillin. In 1990s methicillin resistant *Staphylococcus aureus* (MRSA) became common. Thus, there is a need to look for newer and newer antimicrobial substance for controlling pathogenic microorganisms. It has also been suggested that the herbal medicines used by different ethnic people can provide a way out for developing newer antimicrobial substance which would be safe and efficient.

Plants have formed the basis of traditional medicine practices that have been used for thousands of years by people in China, India, and many other countries especially developing and under developed countries of the world. Some of the earliest records of the usage of plants as drugs are found in *Artharvaveda*, which is the basis for Ayurvedic medicine in India (dating back to 2000 BC), the clay tablets in Mesopotamia (1700 BC), and the Eber Papyrus in Egypt (1550 BC). Charak and Sushruta in 600 BC identified some ferns with medicinal properties, of which *Dryopteris filixmas*, the male fern, is used as vermifuge. Several Indian workers like Chopra (1933), Puri and Arora (1961) have mentioned many ferns having medicinal properties.

Pteridophytes represent an important part of the Indian flora. The pteridophytic population of political India is about 10% of the total pteridophytic flora of the world (Frazer Jenkins, 2008). It has been pointed out that pteridophytes are used by ethnic people as medicinal plants (Dixit, 1974).

The Barak Valley comprises of Cachar, Karimganj, and Hailakandi district of Southern Assam, India. Beddome (1892) reported a total of 25 species of pteridophytes from the then Cachar District and Sylhet District of Assam. However, the present Karimganj district is only a part of erstwhile Sylhet, most of which has been annexed with East Pakistan (now Bangladesh) when India was partitioned in 1947. Hailakandi district is a part of erstwhile Cachar. In last part of 20th century a renewed interest was observed among local workers to study fern flora of Southern Assam. They have studied fern flora of Karimganj (Bhattacharya, 1990; 1994, Bhattacharya *et al.*, 2002; Dutta Choudhury, 1997), Cachar (Bhattacharya *et al.*, 1998), Hailakandi (Dutta Choudhury and Bhattacharya, 1997). There are some other papers also (Nath and Bhattacharya, 2002, Sen and Bhattacharya, 2007). Dey *et al.*, (2011) attemptedto make a consolidated flora offerns found in Barak Valley by making a review of different species which have been reported so far from the area in which they accepted 159 species, including two varieties, in 65 genera under 37 families as the fern flora of the Barak

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Valley. In some cases, where further work is necessary to clear confusion, it has been indicated stating reasons for the same. Thus it is interesting to see that out of about 1000 species of pteridophytes in India, 355 species are from Assam and 159 species are found in southern part of the Assam. This shows the rich diversity of fern flora in the area. More interestingly Dutta Choudhury *et al.*, (2009) mentioned a total of 108 species (actually, slightly less than that because some synonyms have been treated as separate species) of pteridophytes from the valley, out of which 56 species are ethno-medicinally important. Thus, the rich diversity of fern flora and their uses in ethno medicine attracted the present workers to study the antibacterial activity of the ferns found in Barak valley.

In the present study, some ethnomedicinal ferns of Karimganj were screened for their antibacterial activity. It may be stated here that many species could not be used for antibacterial screening because their number has much reduced in the wild and therefore enough material for preparation of extract could not be obtained.

MATERIALS AND METHODS

Survey of ethnobotanical data:

For survey of ethnomedicinal importance of pteridophytes the available literatures were consulted. A total of 21plants were selected on the basis of their availability and record of usefulness. These were tabulated (see Appendix) showing the uses as folk medicine described by different authors.

Collection of plant materials

Fresh plant material was collected from Karimganj district of Barak Valley for a period of 29 months i.e., December, 2012 to April, 2015. The collected plants were cleaned under tap water and were allowed to shade dry.

Taxonomic treatments

One set of collected plants were used for identification and the other set was used for herbarium sheets. The identifications were made by critical examination of herbarium specimens at Central National Herbarium (CAL) and published relevant literature (Beddome, 1865-1870; Borthakur *et al.*, 2001; Fraser Jenkins, 2008). Set of identified specimens have been submitted in Karimganj College Herbarium for further study.

Preparation of plant extract

The shade dried plants were then grinded to powder. Five gram of each plant powder was dipped in 20 ml of four different solvents i.e., methanol, acetone, and petroleum ether for 72 hours. After 72 hours the extract was filtered through Whatman filter paper no. 1. The extract was then left in the room temperature so that the solvent could evaporate. But in case of water extract, it was prepared by boiling method. The dried plant extract was then stored in a refrigerator at 4°C.

Bacterial strains

The test organisms were collected from the culture collection of the Institute of Microbial Technology (IMTECH) Chandigarh. These includes *Bacillus subtilis* (MTCC NO. 736), *Escherichia coli* (MTCC NO. 1610), *Pseudomonas aeruginosa* (MTCC NO. 3541), *Staphylococcus aureus* (MTCC NO. 3160), *Proteus vulgaris* (MTCC NO. 1771), The bacteria were sub-cultured on nutrient agar (HIMEDIA M0010100G) plates and incubated at 37°C for 24 hours and stored at 4°C in the refrigerator to maintain stock culture.

Maintenance of bacterial strains

Organisms were maintained at -80°C and -20°C refrigerators in a glycerol stock.

The Inoculumn

The inoculum for antibacterial assay was prepared by inoculating freshly grown bacterial culture in normal saline solution and the turbidity was matched with 0.5 McFarland Standard.

The medium

Mueller-Hinton agar medium was employed for disc diffusion antibacterial testing. Dehydrated medium supplied by Sisco Research Laboratories (SRL) Pvt. Ltd., Mumbai was used. The medium contained per litre, beef infusion from 300 g beef, acid hydrolysate of casein 17.5 g, starch 1.5 g, and agar 17 g. The medium was prepared by dissolving the dehydrated mixture of ingredients in distilled water. After boiling, pH was adjusted to 7.4 and sterilised by autoclaving at 121°C for 15 minutes. The medium was poured in Petri plates.

***In vitro* antibacterial assay**

The Mueller-Hinton agar plates were seeded with bacterial inoculum aided with sterile swab and was allowed to dry for 30 minutes. Then over the plates sterile filter paper discs were placed, which were impregnated with 10µL of each plant extract (500 mg/500µl of solvent). For negative control discs impregnated with each four different solvents was used. The plates were then incubated at 37°C for 24 hours. The inhibition zone was measured from 6mm to 14mm.

RESULTS and DISCUSSION

The traditional system of medicine has been in use since the dawn of civilization. For thousands of years plants have been used for healing different ailments. Even today tribals and different other communities in remote areas use ethno medicinal plants for primary health care and also for treating certain chronic diseases.

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Ayurveda currently utilizes as many as hundred single drugs and over eight thousand compound formulations of recognized merit (Zahin *et al.*, 2010).

In the present work ethnomedicinal uses of 21 ferns were reviewed from available literature and antibacterial activity of leaf extract of those plants were studied using four solvents viz. petroleum ether, acetone, methanol, water against five potentially pathogenic micro-organisms viz. *Bacillus subtilis, Escherichia coli, Proteus vulgaris, Pseudomonas aeruginosa.* Among the 21 species, 10 ferns showed antibacterial activity of their leaf extract. The ferns having antibacterial activity are *Adiantum capillus-veneris, Asplenium nidus, Cyathea brunoniana, Drynaria quercifolia, Pityrogramma calomelanos, Dipteris wallichii, Lygodium flexuosum* and *Lygodium japonicum*. This shows huge potential in the ferns to be used for controlling microorganisms (Table 1).

The leaf extract of *Adiantum capillus-veneri*s L. is used against fever cough and bronchial disorders (Dixit, 1984). Chopra *et al.*, (1956) and Jain (1991) also reported the same use. Kumar *et al.*, (2003) reported that the plant hasantibacterial, antifungal and antiviral properties. In the present experiment, the acetone extract of the leaf was found active against *Staphylococcus aureus* (8 mm) and *Bacillus subtilis* (inhibition zone diameter 6 mm). Under the family Adiantaceae only one species *Adiantum capillus-veneris* has been tried in the present experiment. The acetone extract of the plant showed antibacterial activity against two Gram positive organisms viz. *Bacillus subtilis* and *Staphylococcus aureus*. However, methanol extract showed inhibition zone against Gram negative bacteria *Escherichia coli* (7 mm). It is important to note that Guha (Ghosh) *et al.,* (2005) reported antifungal activity of *Adiantum capillus-veneris* and considered it better than *Adiantum lunulatum.*

Ethnomedicinal uses of extract obtained by crushing leaves of *Asplenium nidus* L. locally to cure skin diseases (Dutta Choudhury *et al.*, 2009). The plant is used medicinally to cure sores and ulcers and also as depurative and sedative (Dutta Choudhury *et al.*, 2009). The present work shows that ethanol extract of *Asplenium nidus* L. is very effective against all the test micro-organisms except for *Bacillus subtilis*. But, this plant has become threatened in its natural habitat in Barak Valley and only few plants are available in some restricted areas. Therefore, extraction with petroleum ether, acetone, methanol and water were avoided. Only ethanol extract was tried against the test organisms. It may be noted that *Asplenium*'s leaf extract produced good inhibition zones against *Staphylococcus aureus* (10 mm), *Escherichia coli* (8 mm), *Pseudomonas aeruginosa* (12 mm) and *Proteus vulgaris* (7mm). Only one leaf of this plant was used for this experiment. The present authors decided not to pursue further investigation with this endangered plant.

Similarly, *Drynaria quercifolia* (L.) J. Sm. was reported to have antibacterial compound in rhizome (Khan *et al.*, 2007). In the present experiment it has been found that *Drynaria quercifolia* (L.) J. Sm. leaf extract exhibit antibacterial activity against *Pseudomonas aeruginosa* and *Proteus vulgaris* when petroleum ether and acetone were used as solvents. Acetone extract also produced inhibition zone against *Staphylococcus aureus*. Methanol extract was affective against all test organisms. Irudayaraj and Santhamarai (2004) also reported antimicrobial activity in the ethanol extract of the rhizome of *Drynaria quercifolia* (L.) J. Sm. against *Candida albicans, Escherichia coli, Klebsiella pneumonia, Staphylococcus aureus* and *Pseudomonas aeruginosa*. Kandhasmy *et al.*, (2009) reported ethanolic and methanolic extract of rhizome of *Drynaria quercifolia* (L.) J. Sm. was having antibacterial activity against wide range of micro-organisms. Present report demonstrates that the activity is not restricted in rhizome but present in leaf also.

Among the species showing antibacterial activity of leaf extract the family Cyatheaceae deserves special mention. The ethnomedicinal use of *Cyathea gigantea* (Wall *ex* Hook.) Holt. and *Cyathea brunoniana* Clarke is very interesting. It has been reported that soft and young caudex of the plant is cut into pieces and crushed in a morter and added to water to make a paste which is then applied locally on cuts and wounds for immediate clotting of blood. The same is also used to check microbial growth in cut surfaces to prevent formation of abscess. Fresh paste is applied once a day till complete healing (Dutta Choudhury *et al.*, 2009). In case of *Cyathea brunoniana* Clarke apical soft portion of the caudex cut into pieces and crushed in a mortar and added water to make a paste. The paste is then applied locally on major cuts or wounds for immediate clotting of blood. The same also prevent microbial growth in cut surface so that no abscess could develop. Fresh paste is applied everyday till the wound is healed (Shil and Dutta Choudhury, 2009). The leaf extract in both acetone and methanol were effective against *Pseudomonas aeruginosa, Staphylococcus aureus*, and *Proteus vulgaris*. The methanol extract was also affective against *Bacillus subtilis.* Similarly, pinnule extract of *Cyathea gigantea* was found to be active against *Bacillus subtilis, Pseudomonas aeruginosa,* and *Staphylococcus aureus* when acetone, methanol and water were used as solvents.

The acetone, methanol and water extract of *Dipteris wallichii* (R.Br.) Moore was found to be active against *Bacillus subtilis* and *Proteus vulgaris.* Water extract was found to be active against *Pseudomonas aeruginosa*. Fresh rhizome of *Dipteris wallichii* (R.Br.) Moore is cut into pieces pounded and made a paste. The paste is then diluted to double its contents and decanted. The decoction is taken internally (1/2 cup or 20 ml) a day in empty stomach for the treatment of jaundice. The process

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Table 1: Inhibition zone diameter (mm) shown by fern leaf extract and antibiotic against the test organisms

Sl. No.	Ferns (Herbarium sheet No)	Solvent	*Bacillus subtilis*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Proteus vulgaris*
1.	*Adiantum capillus-veneris* L (Call No M782).	Petroleum ether	-	-	-	-	-
		Acetone	6	-	-	8	-
		Methanol	-	7	-		-
		Water	-	-	6	-	-
2.	*Aspleniumnidus*L.	Ethanol	-	8	12	10	7
3.	*Blechnum orientale* L. (Call No M783).	Petroleum ether	-	-	-	-	-
		Acetone	-	-	-	-	-
		Methanol	-	-	-	-	-
		Water	-	-	-	-	-
4.	*Cheilanthes albomarginata* C.B. Clarke (Call No M784).	Petroleum ether	-	-	-	-	-
		Acetone	-	-	-	-	-
		Methanol	-	-	-	-	-
		Water	-	-	-	-	-
5.	*Cyathea brunoniana* (Wall.*ex* Hook.) C.B. Clarke & Baker (Call No M785).	Petroleum ether	-	-	-	-	-
		Acetone	-	-	6	6	8
		Methanol	7	-	11	10	12
		Water	-	-	-	-	-
6.	*Cyathea gigantean* (Wall. *ex* Hook.)Holttum (Call No M786).	Petroleum ether	6	-	-	-	-
		Acetone	9	-	10	14	-
		Methanol	6	-	8	8	-
		Water	6	-	7	6	-
7.	*Dicranopteris linearis* (Burm. f) Underw. (Call No M787).	Petroleum ether	-	-	-	-	-
		Acetone	-	-	-	-	-
		Methanol	-	-	-	-	-
		Water	-	-	-	-	-
8.	*Dipteriswallichii* (R. Br.) T. Moore (Call No M788).	Petroleum ether	-	-	-	-	-
		Acetone	12	-	-	-	13
		Methanol	8	-	-	-	11
		Water	12		11		9
9.	*Drynaria quercifolia* (L.) J. Sm. (Call No M789).	Petroleum ether	-	-	6	-	6
		Acetone	-	-	6	8	7
		Methanol	6	7	8	7	6
		Water	-	-	-	-	-
10.	*Helminthostachys zeylanica* (L.) Hook. (Call No M790).	Petroleum ether	-	-	-	-	-
		Acetone	-	-	-	-	-
		Methanol	-	-	-	-	-
		Water	-	-	-	-	-
11.	*Lindsaea ensifolia* Sw. (Call No M791).	Petroleum ether	-	-	-	-	-
		Acetone	-	-	-	-	-
		Methanol	-	-	-	-	-
		Water	-	-	-	-	-
12.	*Lygodiumflexuosum* (L.) Sw. (Call No M792).	Petroleum ether	-	7	-	-	-
		Acetone	-	-	-	-	-
		Methanol	9	-	-	8	-
		Water	-	-	-	-	-
13.	*Lygodium japonicum* (Thunb.) Sw. (Call No M793).	Petroleum ether	-	-	-	-	-
		Acetone	-	-	-	8	-
		Methanol	7	-	-	-	-
		Water	-	-	6	-	8
14.	*Microsorum punctatum* (L.) Copel. (Call No M794).	Petroleum ether	-	-	-	-	-
		Acetone	-	-	-	-	-
		Methanol	-	-	-	-	-
		Water	-	-	-	-	-

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Table 1: (Continued) Inhibition zone diameter (mm) shown by fern leaf extract and antibiotic against the test organisms.

Sl. No.	Ferns (Herbarium sheet No)	Solvent	*Bacillis subtilis*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Proteus vulgaris*
15.	*Pityrogramma calomelanos* (L.) Link (Call No M795).	Petroleum ether	-	-	-	-	-
		Acetone	6	-	-	7	7
		Methanol	8	-	-	9	-
		Water	-	-	-	-	-
16.	*Pronephrium nudatum* (Roxb. *ex* Griff) Holttum (Call No M796).	Petroleum ether	-	-	-	-	-
		Acetone	-	-	-	-	-
		Methanol	-	-	-	-	-
		Water	-	-	-	-	-
17.	*Pteris biaurita* L. (Call No M797).	Petroleum ether	-	-	-	-	-
		Acetone	8	-	7	-	-
		Methanol	-	-	-	6	-
		Water	-	-	-	-	-
18.	*Pyrrosia adnascens* (Sw.) Ching (Call No M798).	Petroleum ether	-	-	-	-	-
		Acetone	-	-	-	-	-
		Methanol	-	-	-	-	-
		Water	-	-	-	-	-
19.	*Sphenomeris chinensis* (L.) Maxon (Call No M799).	Petroleum ether	-	-	-	-	-
		Acetone	-	-	-	-	-
		Methanol	-	-	-	-	-
		Water	-	-	-	-	-
20.	*Stenochlaena palustris* (Burm. f.) Bedd. (Call No M800).	Petroleum ether	-	-	-	-	-
		Acetone	-	-	-	-	-
		Methanol	-	-	-	-	-
		Water	-	-	-	-	-
21.	*Vittaria elongate* Sw. (Call No M801).	Petroleum ether	-	-	-	-	-
		Acetone	-	-	-	-	-
		Methanol	-	-	-	-	-
		Water	-	-	-	-	-
	Ciprofloxacin	(5 micro gram)	24	24	20	20	28

is continued till recovery (Dutta Choudhury and Choudhury, 2002). Further decoction of rhizome is taken internally in empty stomach for treating hepatitis. The drug is given for seven days for getting cured. (Dutta Choudhury *et al.*, 2009).

Pteris biaurita L. and *Pteris ensiformis* Burm.f. are one of the most popular ethnobotanically important species in Taiwan (Salatino and Prado, 1998). They are anti tumour and antibacterial in activity. (Tanaka *et al.*, 1978). In the present experiment acetone extract of *Pteris biaurita* L. produced inhibition zone against *Bacillus subtilis* (8mm) and *Pseudomonas aeruginosa* (7mm). Methanol extract showed inhibition zone only against *Staphylococcus aureus* (6mm). The ethnoclaims for this species include *1*. fronds are antibacterial, which are made into a paste and applied in wounds (Singh, 1999) and *2*. rhizomes are used in treatment of wounds. (Borthakur *et al.*, 2001). These claims are supported by the finding of the present investigation.

In the present work, silver fern *Pityrogramma calomelanos* (L.) Link. was found to be active against *Bacillus subtilis*, *Staphylococcus aureus* and *Proteus vulgaris* when acetone extract of leaf was used. Methanol extract of leaf showed antibacterial activity against two Gram positive organisms viz. *Bacillus subtilis* and *Staphylococcus aureus*. If we study ethoclaims in favour of this species, we find that one of the use is tea prepared out of the frond is used as a cure for flu, fever and cough. (Borthakur *et al.*, 2001).

Among Lygodiaceae, *Lygodium flexuossum* L. plants are used as expectorant; rhizomes boiled with mustard oil and locally applied on carbuncles and in rheumatism, sprains, scabies, ulcers, eczema and cuts. The aqueous extract of the rhizome is used to cure gonorrhea. It has been observed in the present experiment that leaf extract of *Lygodium flexuossum* L. in petroleum ether showed inhibition zone against *Escherichia coli* (7 mm), whilemethanol extract of the leaf of the same species produced inhibition zone against both *Bacillus subtilis* (8 mm) and *Staphylococcus aureus* (9 mm). Leaf decoction of *Lygodium japonicum* (Thunb.) Sw. is given indysentery. It is used as one of the ingredients in many lotions. Leaves are applied in the form of poultices for skin

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diseases and swelling. Crushed leaves are used to cure high cough (Manickam *et al.*, 1992). In the present experiment *Lygodium japonicum* (Thunb.) Sw. leaf extract showed inhibition zone as follows: for acetone extract against *Staphylococcus aureus* (8 mm), for methanol extract against *Bacillus subtilis* (7 mm) and for water extract against *Proteus vulgaris* (8mm) and *Pseudomonas aeruginosa* (6mm).

There has not been any inhibition zone shown with extracts of *Blechnum orientale, Dicarnopteris linearis, Helminthostachys zeylinica, Microsorum punctatum, Pronephrium nudatum, Pyrrosia adnascens, Spenomeris chinensis, Stenochlaena palustris, Tectaria vasta* (Blume) Copel, and *Vittariae longata*. Ethno-medicinal uses are known for these species also. But in the present work there has not been any antibacterial activity shown by these species.

The ferns like *Asplenium nidus* L., *Cyathea brunoniana* Clark, *Dipteris wallichii* (R.Br.) Moore etc. are fast disappearing in the valley. It is important that our civilization should understand importance of these ferns and take immediate step to conserve them.

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Appendix: List of ferns selected for antibacterial screening and their ethnomedicinal importance obtained in survey of literature. **Asterisks (*)** indicate ferns that did not show antibacterial activity.

1. ***Adiantum capillus-veneris*** L.
   1. Fronds are used in respiratory problems and menstrual disorders (Sen and Ghosh, 2011).
   2. The leaf extract is used for fever, cough and bronchial disorders (Dixit, 1984).
   3. Used as a stimulant, purgative, emollient, tonic and hair tonic. It has anti-cancerous, hypoglycaemic, aphrodisiac, antifungal, antibacterial and antiviral properties (Kumar *et al.*, 2003).
   4. Plant is used in the treatment of diuretic and astringent (Perumal, 2010).
   5. It is used in skin diseases, diabetes, cough and fever (Chopra *et al.*, 1956; Jain, 1991).
   6. Fronds used as expectorant, diuretic and tonic, febrifuge; whole plant is steamed for small pox cure. Fronds used in imposthumes of uterus, hard swelling and tumors of spleen; fronds are used to treat bronchitis, cold, cough, fever and menstrual irregularities (Dutta Choudhury *et al.*, 2009).
   7. Infusion with sugar or honey is given in catarrhal infections and menstrual discharge. Plant is used as expectorant (Caius, 1935).
   8. Rhizome are expectorant and also given in difficult respiration, used in cold imposthumes of uterus hard swelling and hard tumors of spleen (Asolkar *et al.*, 1992).
2. ***Asplenium nidus*** L.
   1. Extract obtained by crushing leaves applied locally to cure skin diseases (Dutta Choudhury *et al.,* 2009).
   2. The plant is used medicinally to cure sores and ulcers and also as depurative and sedative. (Dutta Choudhury *et al.*, 2009).
3. ***Blecnum orientale*** L.*
   1. Hot decoction of pinnae is applied externally over abscess to liberate pus and also for its antiseptic action. Fresh decoction is applied once days till abscess dry up (Shil and Dutta Choudhury, 2009).
   2. Rhizome and fronds are pounded and applied on cuts and wounds for clotting of blood and hasten its healings by Nishi tribe (Rawat and Chowdhury, 1998).
   3. It is used as poultice for boils in Malay. Rhizome is used as anthelmintic in China. Rhizome used as urinary disorders as cure of sanipat (delirium) (Rawat and Chowdhury, 1998).
   4. Fronds are antibacterial, given for the treatment of fever, skin diseases, throat and gastric ulcers. Leaves and rhizomes are used as a cooling agent and in the treatment of burns and ulcers (Singh, 1999).
   5. Hot decoction of the pinnae applied externally over abscess to liberate pus and also for antiseptic action (Dutta Choudhury *et al.*, 2009).

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4. ***Cheilanthes albomarginata*** C.B. Clarke*
   1. Plant juice is used in tuberculosis (Das and Dutta Choudhury, 2012).
   2. Whole plant extract is used in cuts and wounds as antiseptic (Dutta Choudhury *et al.*, 2009).
   3. Young plants are used in making tonic. Extract missed with honey is taken after meal by person suffering from weakness due to tuberculosis (Dutta Choudhury *et al.*, 2009).
5. ***Cyathea brunoniana*** (Wall. *ex* Hook.) C.B. Clarke & Baker
   1. Apical soft portion of the caudex cut into pieces and crushed in a morter and added water to make a paste. The paste is then applied locally on major cuts or wounds for immediate clotting of blood. The same also prevent microbial growth in cut surface so that no abscess could develop (Shil and Dutta Choudhury, 2009).
6. ***Cyathea gigantea***(Wall. *ex* Hook.) Holttum
   1. Soft and young caudex of the plant is cut into pieces and crushed in a mortar and added to water to make a paste which is then applied locally on cuts and wounds for immediate clotting of blood. The same is also used to check microbial growth in cut surfaces to prevent formation of abscess. (Dutta Choudhury *et al.*, 2009)
   2. Arial part made into paste is used as anti-inflammatory agent (Borthakur *et al.*, 2001).
7. ***Dipteris wallichii*** (R. Br.) T. Moore
   1. Fresh rhizome is cut into pieces pounded and made a paste. The paste is then diluted to double its contents and decanted. The decoction taken internally (20 ml) a day in empty stomach for the treatment of jaundice (Dutta Choudhury and Choudhury, 2002).
8. ***Dicranopteris linearis*** (Burm. f) Underw.*
   1. Freshly extracted fronds juice is slightly heated and the decoction is taken internally during throat pain (Shil and Dutta Choudhury, 2009).
   2. Fronds and rhizome is used in asthma, women's sterility. It is also an antihelmintic (Perumal, 2010).
   3. Decoction given to improve fertility in sterile women (Das and Dutta Choudhury, 2012).
9. ***Drynaria quercifolia*** (L.) J. Sm.
   1. The rhizome paste mixing with molasses taken internally during cardiac problem (Shil and Dutta Choudhury, 2009).
   2. Paste obtained by crushing rhizome applied externally in the form of poultice on fractured bones after setting up the bones (Shil and Dutta Choudhury, 2009).
   3. Rhizome paste is applied externally in blood coagulation (Shil and Dutta Choudhury, 2009).
   4. Whole plant, rhizome and root are used to cure fever, dyspepsia, cough and swellings. It is also antihelmintic and astringent (Perumal, 2010).
   5. Rhizome is bitter and antibacterial, laxative and anti-inflammatory. Used against typhoid, cough, diarrhea etc (Deepa *et al.*, 2009).
   6. Decoction used to get relief from gastritis (Das and Dutta Choudhury, 2012).
   7. Rhizome is used to treat bone fracture, cough, headache and typhoid fever. The fronds are administered for treatment fever, dyspepsia and cough in Malaysia. In India, it is used in the treatment of body ache, rheumatism, skin diseases, and as a tonic, expectorant and antihelmintic (Asolkar *et al.*, 1992).
   8. In Barak Valley of Southern Assam, it is commonly called as Uphatkarul and is used to treat tuberculosis and throat infections (Sen and Bhattacharya, 2007).
   9. An extract of the rhizome is used to treat eye infections. Mixed with other plants it is used to cure gonorrhea (Giesen *et al.*, 2007).
   10. Rhizome is used as astringent, fronds used in phthisis, hectic fever, dyspepsia, and cough. In Malayasia it is used as poultice on swellings; the decoction of the plant is used in typhoid fever by Vaidyas; the fluid extracted from the fronds show antibacterial properties (Chopra *et al.*, 1956; Dixit *et al.*, 1984).
   11. The plant is used in the treatment of body ache, rheumatism, and skin diseases and as tonic, expectorant and anthelmintic; fronds are antibacterial and used to treat swellings; rhizome is used to treat bone fracture. Cough, headache and typhoid fever (Jain, 1991; Asolkar *et al.*, 1992).
   12. Rhizomes of the plant are used as astringent; fronds used in hectic fever, dyspepsia and cough. The decoction of the plant is used in typhoid fever. The fluid extracted from the fronds has anti- bacterial properties. The plant is used in the treatment of body ache, rheumatism, skin diseases and as tonic, expectorant and anthelmintic. (Dutta Choudhury *et al.*, 2009).
   13. The whole plant is anthelmintic, pectoral expectorant and tonic. The plant is used in chest and skin diseases and also for loss of appetite. The rhizome is used in typhoid and frond as poultice in swellings (Kaushik and Dhiman, 1995).
10. ***Helminthostachys zeylanica*** (L.) Hook.*
    1. The fronds are reported to be aperients, intoxicant, and anodyne; also used in sciatica, as an antiviral, antipyretic, anti-inflammatory and intoxicant (Chopra *et al.*, 1956; Dixit and Vohra, 1984).
    2. The rhizome is used in dysentery, cataract, sciatica, malaria and also as a tonic and mild aperients. A decoction of the plant is given for curing impotency and the juice of the leaves is used to relieve blisters on the tongue (Jain *et al.*, 1991; Ambasta, 1986).
    3. The decoction of rhizome is used for the treatment of impotency, whooping cough, phthisis. In combination with the roots of *Chlorophyllum tuberosum* and roots of *Bombaxceiba* made into a paste when applied for one month to relieve waist pain and used also as a tonic. A paste of the rhizome, curd and crushed termite is known to promote strength and vitality. The powder of the rhizome is given for spermatorrhoea and for improving memory power (Singh, 1999; Singh and Maheshwari, 1995).
    4. Kottunaikan tribes of Kerala (Malappuram district) use the plant against snake bite. Tender stalks of the plant are eaten in Malayasia. Young leaves are used in salad and as vegetables in Olhilippines. Rhizome used against dysentery, malaria and also as tonic and as mild aperients. Leaf juice relieves blisters on tongue (Mathew *et al.*, 1999).
    5. Intoxicant, anodyne and used against sciatica (Anonymus, 1999).
    6. Rhizome is used as a brain tonic (Trivedi, 2002).
    7. The fronds are aperients (Chopra *et al.,* 1969; Dixit and Vohra, 1984).
    8. Intoxicant, anodyne, also used in sciatica, malaria and also as tonic and mild aperients. Leaf juice relieves blisters on tongue and rhizome is used to treat impotency and jaundice (Ambasta, 1986; Jain, 1991; Asolkar *et al.*, 1992).
    9. The young fronds and fleshy rhizomes are eaten in Gorakhpur, Garhwal and Assam; the fronds are aperients, intoxicant, anodyne and used in sciatica; young leaves are used as salad or cooked as vegetable in Philippines; tender stalks eaten in Malaya; rhizome used in dysentery, cataract, sciatica, malaria and also as tonic and mild aperients (Chopra *et al.*, 1956; Chopra *et al.*, 1969; Dixit and Vohra, 1984).
    10. Leaf juice relieves blisters on tongue and rhizome is used to treat impotency and jaundice (Ambasta, 1986; Jain, 1991; Asolkar *et al.*, 1992).
    11. Powdered rhizome along with cow milk is used for vitality and brain tonic (Singh *et al.*, 1980).
    12. The plant is used to cure impotency (Kholia and Punetha, 2005).

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11. ***Lindsaea ensifolia*** Sw.*
    Leaves are used internally for chronic enteritis in Mauritius (Dixit, 1984).
12. ***Lygodium flexuosum*** (L.) Sw.
    1. One teaspoonful of leaf powder is mixed in milk and given orally for children to improve memory (Thulsi Rao *et al.*, 2007).
    2. Leaf paste is applied on boils (Das and Dutta Choudhury, 2012).
    3. Rachis of the plant is tied over forehead to reduce headache by the Reang tribe (Dutta Choudhury *et al.*, 2009).
    4. The plants are used as expectorant; rhizomes boiled with mustard oil and locally applied on carbuncles and in rheumatism, sprains, scabies, ulcers, eczema and cuts. The aqueous extract of the rhizome is used to cure gonorrhoea. The paste of the rhizome is applied on piles and the rhizome is also tied on the waist. Rhizome is used as appetizer; it is also used for treatment of abdominal pain, cholera, cuts, eczema, indigestion, jaundice, scabies and ulcers, the plant is also used in pleurisy (Borthakur *et al*., 2001; Kaushik and Dhiman, 1995).
13. ***Lygodium japonicum*** (Thunb.) Sw.
    1. Leaf decoction is given in dysentery. It is used as one of the ingredients in many lotions. Leaves are applied in the form of poultices for skin diseases and swelling. Crushed leaves are used to cure hiccough (Manickam *et al.*, 1992).
    2. Rhizome is taken orally mixing with water to get cured from food poisoning (Dutta Choudhury *et al.*, 2009).
    3. The plant is used as expectorant; decoction of vegetative parts and spores is used as diuretic or cathartic. Roots are used as veterinary medicine to treat animal wounds (Borthakur *et al.*, 2001).
    4. The plant is used as expectorant and in haematuria. (Dutta Choudhury *et al.,* 2009).
14. ***Microsorum punctatum*** (L.) Copel.*
    1. About 20g paste obtained by crushing fresh rhizome along with seeds of *Piper nigrum* is taken orally to cure cough and cold. It should be taken thrice a day till the disease is cured.(Shil and Dutta Choudhury, 2009)
    2. Leaves and juice are used as purgative, diuretic and wound healer in Ivory Coast (Boquet, 1974).
    3. The plant is used as antidote to snake bite (Jain, 1991).
15. ***Pronephrium nudatum*** (Roxb. ex Griff) Holttum *
    1. Leaf paste applied on forehead to get relief from headache (Das and Dutta Choudhury, 2012).
    2. Cold decoction of pinnae is used as mouth wash during acute pyorrhea. 2-3 washes given a day till it is cured (Dutta Choudhury and Choudhury, 2002).
    3. Cold decoction of pinnae is used for acute pyorrhea, as mouth wash (Dutta Choudhury *et al.*, 2009).
16. ***Pteris biaurita*** L.
    1. The fronds are antibacterial, which are made into a paste and applied in wounds (Singh, 1999).
    2. Rhizomes are used in treatment of wounds. (Borthakur *et al.*, 2001).

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