

13. Testing of Local Herbal Products for Fish/Shrimp Disease Prevention and Control

13.1 Executive Summary

Experiments were conducted on selection of medicinal plant products, preparation of extracts (crude, semi-crude and fine), selection and collection of fish species, isolation of bacteria and use of medicinal plant extracts on disease recovery of fishes. Twelve medicinal plant products, six crude extracts, two semi-crude extracts and four fine extracts were collected and prepared to observe their effect on disease recovery of fish. Diseased shing were collected from Talha Soha Fish Farm, Muktagacha and bacteria were isolated from the fishes up to genus. An experiment was conducted to observe disease recovery of sarpunti and rui treated with neem seed oil, kalojira seed oil, neem leaf extract and mehagoni seed extract for 28 days. Neem seed oil extracts exhibited satisfactory recovery in third week with 6 ml/kg feed dose. Disease fish treated with kalojira seed oil showed the best performance in respect of disease and wound recovery. Better recovery were found in third week at a dose of 4 ml/kg feed and 6 ml/kg feed. However, fishes treated with neem leaf extract and mehagoni seed oil extract showed poor performance in respect of disease recovery of fishes. Among doses 6 ml/kg feed exhibited the best performance followed by 4 ml/kg feed.

13.2 Background and Justification

Chemotherapy has progressed internationally for treating the most diversified infectious disease of fish (Shieszko 1959). However, there are problems associated with the use of such chemicals. Thus it is the demand of the time to look for alternative means of commercial synthetic drugs. Herbalism (or "herbal medicine") is use of plants for medicinal purposes. Herbal medicine or phytochemicals have antiviral, antibacterial, antifungal and antihelminthic properties. Plants have been the basis for medical treatments through much of human history, and such traditional medicine is still widely practiced. Archaeological evidence indicates that the use of medicinal plants dates at least to the Paleolithic, approximately 60,000 years ago. More than two thirds of the world's plant species-at least 35,000 of which are estimated to have medicinal value. Herbal products having immune medicinal value could be an alternative means.

Medicinal plants, the natural drugs are used to regain the alternations made in normal physiological system by foreign organisms or by malfunctioning of the body (Sharma *et al.* 2009). Medicinal plants are vital source of drugs from the ancient time holding the scenario of the Indian system of medicine (Sharma *et al.* 2009). According to Ghoni (1998) medicinal plants are rich sources of bioactive compounds and thus serve as important raw materials for drug production. In Bangladesh, different kinds of medicinal herbs are available which grow in roadside, small jungles are fellow lands and most of them are cultivable with very low cost. Many species of these herbs are used as directly human food or as medicine such as, *Andrographis paviculata*, *Azadirachta indica*, *Basella alba*, *Allium cepa*, *Allium sativum*, *Calotropis gigantean* and *Monordica chorantia* (Muniruzzaman & Chowdhury 2004).

Aquaculture is one of the important sectors contributing significantly in the national economy of Bangladesh. For increased production and profit, fish farmers are encouraged towards intensification of culture system. In such practice of fish/shrimp farming, disease becomes major problem. Disease is one of the most important problems of fish production both in culture system and wild condition of Bangladesh (Rahman & Chowdhury 1996). Fishes have been suffering from many diseases such as Epizootic ulcerative syndrome (EUS), tail and fin rot, fungal, parasitic and bacterial infections (Chowdhury *et al.* 1999).

With the outbreak of EUS in 1988, *Channa* sp., *Puntius* sp., *Anabas* sp., *Clarias* sp., and other indigenous species of fish are seriously affected (Barua *et al.* 1991). External and internal parasites damage organs resulting necrosis, pyknosis, hemorrhage, hypertrophy, hyperplasia and other changes causing serious harm to host (Ahmed & Banu 2001). Outbreak of a viral disease, White spot syndrome (WSS) or simply white spot has been known to play a major role to cause drastic losses in shrimp production of the coastal region of Bangladesh (Chowdhury & Muniruzzaman 2003). Thus to prevent and control of fish and shrimp diseases, treatment trials has become an essential component of fish and shrimp production.

In aquaculture, the external inputs required for successful fish production is chemical, which has been used in various farms for centuries (Subasinghe *et al.* 1996). Chemotherapy has progressed internationally for treating the most diversified infectious

disease of fish (Shieszko 1959). However, there are problems associated with the use of such chemicals. Thus it is the demand of the time to look for alternative means of commercial synthetic drugs. Herbal products having immune medicinal value could be an alternative means.

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13.2.1 Medicinal plants and herbs

Plants and herbs have medicinal value and are used to treat different diseases both in home and abroad (Table 68). Few common medicinal plants and herbs and their uses are listed below:

Table 68 List of Medicinal Plants and Herbs and their common uses

Sl	English and Scientific Name	Use/ Treatment
1	Blackberry (<i>Rubus villosus</i>)	Treat sore throat
2	Black Cohosh (<i>Cimicifuga racemosa</i>)	Treat premenstrual discomfort
3	Calendula (<i>Calendula officinalis</i>)	Heal wounds
4	Cayenne (<i>Capsicum annuum</i>)	Prevent peptic ulcers
5	Chamomile, German (<i>Matricaria recutita</i>)	Encourage digestion
6	Cleavers (<i>Galium aparine</i>)	Reduce inflammation
7	Comfrey (<i>Symphytum officinale</i>)	Treat bruises, sprains
8	Crampbark (<i>Viburnum opulus</i>)	Relax muscles
9	Dandelion (<i>Taraxacum officinale</i>)	Diuretic
10	Echinacea (<i>Echinacea purpurea</i>)	Stimulate immune system
11	Elder (<i>Sambucus nigra</i>)	Treat cold symptoms
12	Fennel (<i>Foeniculum vulgare</i>)	Encourage digestion
13	Ginger (<i>Zingiber officinalis</i>)	Treat motion sickness
14	Goldenseal (<i>Hydrastis canadensis</i>)	Reduce inflammation
15	Gumweed (<i>Grindelia spp.</i>)	Treat cold symptoms
16	Hawthorn (<i>Crataegus oxyacanthus</i>)	Promote heart health
17	Marshmallow (<i>Althaea officinalis</i>)	Treat sore throat

SI	English and Scientific Name	Use/ Treatment
18	Mugwort (<i>Artemisia vulgaris</i>)	Stimulate digestion
19	Mullein (<i>Verbascum spp.</i>)	Treat sore throat
20	Nettle (<i>Urtica spp.</i>)	Diuretic
21	Peppermint (<i>Mentha piperita</i>)	Stimulate digestion
22	Pipsissewa (<i>Chimaphila umbellata</i>)	Treat urinary track infections
23	Plantain (<i>Plantago lanceolata</i> or <i>P. major</i>)	Heal wounds
24	St. John's Wort (<i>Hypericum perforatum</i>)	Treat depression
25	Scullcap (<i>Scutellaria spp.</i>)	Ease muscle tension
26	Valerian (<i>Valeriana officinalis</i>)	Sleeping disorders
27	Vitex (<i>Vitex agnus-castus</i>)	Treat premenstrual discomfort
28	Willow Bark (<i>Salix alba</i>)	Treat osteoarthritis
29	Yarrow (<i>Achillea millefolium</i>)	Reduce inflammation
30	Yellow Dock (<i>Rumex crispus</i>)	Stimulate digestion

13.2.2 Poisonous plants

Many food plants possess *toxic* parts, are toxic unless processed, or are toxic at certain stages of their life. Notable examples include:

Apple (*Malus domestica*): Seeds contain cyanogenic glycosides; in most species, the amount found in a single fruit won't kill a person; but it is possible to ingest enough seeds to provide a fatal dose.

Cassava (*Manihot esculenta*): Toxic in the unprocessed form.

Cherry (*Prunus cerasus*), as well as other species (*Prunus* spp) such as peach (*Prunus persica*), plum (*Prunus domestica*), almond (*Prunus dulcis*), and apricot (*Prunus armeniaca*). Leaves and seeds contain cyanogenic glycosides.

Indian pea (*Lathyrus sativus*): A legume grown in Asia and East Africa as an insurance crop for use during famines. Contains oxalyl-L, β -diaminopropionic acid (ODAP), a neurotoxin causing wasting and paralysis if eaten over a long period.

Kidney bean or common bean (*Phaseolus vulgaris*): Contains the lectin phytohaemagglutinin, which causes gastric upset. Toxicity removed by thorough cooking.

Nutmeg (*Myristica fragrans*): Contains myristicin.

Lima bean or Butter Bean (*Phaseolus lunatus*): Raw beans contain dangerous amounts of linamarin, a cyanogenic glucoside.

Lupin: Some varieties have edible seeds. Sweet Lupins have less, and Bitter Lupins have more of the toxic alkaloids lupinine and sparteine.

Onions and garlic: Onions and garlic (genus *Allium*) contain thiosulphate, which in high doses is toxic to dogs, cats and some other livestock.

Potato (*Solanum tuberosum*): Foliage and green-tinged tubers are toxic, containing the glycoalkaloid solanine, which develops as a result of exposure to light. Causes intense digestive disturbances, nervous symptoms, and in high enough doses, death.

Rhubarb (*Rheum rhaponticum*): Leaf blades, but not petioles, contain oxalic acid salts, causing kidney disorders, convulsions and coma.

Tomato (*Solanum lycopersicum*): Foliage and vines contain alkaloid poisons which cause digestive upset and nervous excitement.

13.3 Literature Review

Every parts of the Indian lilac plant (*Azadirachta indica*) are used medicinally (Ghoni 2003). Again, Ruskin (1992) specified that it is effective to against certain fungi of the human body which are difficult to control by synthetic fungicides. But, *Aeromonas hydrophila*, *Pseudomonas fluorescens* and *Myxobacteria* sp. exhibited maximum sensitivity to Aquaneem in terms of percentage reduction of bacterial cell population in comparison of *Escherichia coli* (Das 1999).

The fruit bearing plants guava (*Psidium guajav*) grows wild and also planted commonly as fruit tree in all areas of Bangladesh. Mature fruits are commonly eaten as a nutritious fruits as a source of vitamin C (Ghoni 2003). The plant garlic (*Allium sativum*) is a bulbuous herb with white scale leaves and narrow, angular long green leaves and white flowers in terminal umbels, cultivated all over the Bangladesh as a spice plant (Ghoni 2003). The bulb is the useable part. Garlic counters many infections, including those of the nose, throat and chest. It reduces cholesterol, helps circulatory disorders such as high blood pressure, and lower blood sugar levels (Chevallier 1996). Garlic has a very long folk history of use in a wide range of ailments, particularly ailments such as ringworm, candida and vaginitis where its fungicidal, antiseptic, tonic and parasiticidal properties have proved of benefit (Duke & Ayensu 1985).

The plant turmeric (*Curcuma longa*) is a perennial herb with tufted, large, oblong long-petioled leaves, fragrant flowers in central racemose spikes, cultivated for its rhizomes as a spice crop all over Bangladesh (Ghani 2003). The rhizome of turmeric is effective in the treatment of digestive and liver problems and has been shown to inhibit blood clotting, relieve inflammatory conditions, and help to lower cholesterol levels (Chevallier 1996). The useable part of turmeric rhizome contains an essential oil curcumin and curdione which has anti-cancer properties (Chevallier 1996). Moniruzzaman (2004) found highly inhibitory effect of turmeric on the growth of fish fungal pathogen. According to Ghani (2003), the rhizome of ginger (*Zingiber officinale*) is one of the world's best medicines. It brings relief to digestions troubled by motion sickness and other causes in human beings (Chevallier 1996). The rhizome of *Typhonium trilobatum* is a folklore medicine for snake bite to men (Chopra *et al.* 1996). Again the alcoholic extract of *Bambusa arundinacea* buds was used by injecting to fracture healing in rabbit successfully without any adverse effects (Hoque *et al.* 2004). The kernels of *Derris trifoliata* seed is the most popular for the beautification of lady especially the marriage ceremony. Khan *et al.* (2006) found anti bacterial not anti fungal activity from leaves extracts of *D. trifoliata*. Dark is stimulant, antispasmodic and counter-irritant and is used in rheumatism and dysmenorrhoea and counter-irritant and is used in rheumatism and dysmenorrhoea and also as a fish poison (Chopra *et al.* 1956).

The fruits of the black myrobalan (*Terminalia chebula*) are given internally in the treatment of indigestion, constipation, dysentery, jaundice, piles and painful menstruation and as a general tonic and externally they are used to treat eye discharges and as a local application to chronic ulcers and wounds and as a gargle in stomatitis in human beings (Chopra *et al.* 1956). The leaf henna plant (*Lawsonia inermis*) commonly uses as cosmetic materials (Zumrutdal *et al.* 2008). However, Zumrutdal *et al.* (2008) found in their reviewing literature that many studies showed leaf henna plant has anti tumoural, anti microbial and anti tuberculostatic effect. Again, a poultice of fresh leaves, *Adhatoda zeylanica* is applied to wounds and to inflamed joints in rheumatism (Chevallier 1996). Entire plant of *Leucas aspera* is used as an insecticide and indicated in traditional medicine for coughs, colds, painful swellings and chronic skin eruptions (Chopra *et al.* 2002).

The plumieride has been isolated from the leaves of golden trumpet (*Allamanda cathartica*) which is strong fungitoxicity against some dermatophytes causing dermatomycosis to animals and human beings (Tiwari *et al.* 2002). The Indian sorrel (*Oxalis corniculata*) extract showed cardio relaxant activity on isolated rabbit heart (Achola *et al.* 1995). The Indian coral tree (*Erythrina variegata*) is used as protein supplements to improve the nutritional quality of Maize stover fed to mature goats (Aregheore & Perera 2004). Paste of leaves is applied externally to cure inflammations and to relieve pain in the joints; juice is used to relieve earache and toothache (Ghani 2003). Indian pennywort plant (*Hydrocotyle asiatica*) and Ivy gourd plants (*Coccinea cordifolia*) are exclusively used as medicinal plants and there is no economic use of them. The Indian pennywort plant is useful tonic and cleaning herb for skin problems and digestive disorders and it also strengthens nervous function and memory (Chevallier 1996). The Ivy gourd plants leaves are externally used in skin eruptions (Ghani 2003). Sweet potato (*Ipomoea batatas*) leaves contain at least 15 biologically active anthocyanins that has significant medicinal value for certain human diseases and may also be used as natural food colorants (Islam 2006). Sweet potato leaves decoction is folk remedy for asthma, bug bite, burns, catarrh, diarrhea, fever, nausea, stomach distress and tumor (Osime *et al.* 2008). The leaves of Indian bean (*Lablab purpureas*) are regarded as alexipharmic (antidote to poison) and emmenagogue (Ghani 2003). The Indian bean and sweet potato both are used as vegetable. The banyan trees are planted for shade in the field or village market. The wood is used as low quality fuel. Latex of the banyan tree (*Ficus benghalensis*) is used in relieving pain in toothache and applied to haemorrhoids, warts and aching joint (Chevallier 1996). The ethanol extract of banyan tree sacred (*Ficus religiosa*) bark is antiprotozoal, anthelmintic and antiviral (Ghani 1993). The seed oil of both mustard (*Brassica campestris*) and sun flower (*Helianthus annuus*) are used as vegetable oil. Some compounds of the pollen of *Brassica campestris* possess strong aromatase inhibitory activity (Yang *et al.* 2009). Again, a black cumin (*Nigella sativa*) seed has a high medicinal property (Buriro & Tayyab 2007). Whereas, the leaves of plant sunflower are used in the treatment of malarial fever (Ghani 2003). The banana plant (*Musa paradisaca*) stem juice is used in otalgia and haemoptysis and root possesses anthelmintic properties (Chopra *et al.* 1956). The banana roots are successfully used with

feed in controlling coccidiosis of rabbit in central district of Zimbabwe (Matekaire *et al.* 2005) and juice of stem with feed reduce blood glucose level of diabetic rat (Singh 2007). Mulberry (*Morus indica* L) is non-toxic natural therapeutic agent shown to possess hypoglycemic, hypotensive and diuretic properties (Bondada *et al.* 2001).

The plant crown flower (*Calotropis gigantean*), turpeth root (*Opaculina turpethum*) and *Justicia gendarussa* are exclusively used as medicinal plants. The plant crown flower is moderate to large sized perennial shrub abounding in milky latex, with opposite-decussate oblong-auriculate thick leaves, inodorous purplish which flowers and oblong follicles; grows commonly in the waste lands in all areas of the Bangladesh (Ghani 2003). This plant is used as a traditional medicinal plant (Rastogi & Megrotra 1991) with unique properties (Oudhia & Tripathi 1999). The leaf extract of plant crown flower contains several proteinases as well as calotropoin and other cardiac glycosides. The plant crown flower was used as a traditional medicinal plant (Rastogi & Mehrotra 1991 and Oudhia & Dixit 1994) with unique properties (Oudhia & Tripathi 1998). Traditionally plant crown flower is used alone or with other medicinal plants (Caius 1986) to treat common disease such as fevers, rheumatism, indigestion, cough, cold, eczema, asthma, elephantiasis, nausea, vomiting, diarrhoea (Das 1996). It was also a reputed homeopathic drug (Ghosh 1988, Ferrington 1990). A powder of dried leaves of plant crown flower is an efficacious local application for ulcer, eczema and other skin diseases (Anawer 2001). The plant turpeth root (*Opaculina turpethum*) is a twining or creeping perennial herb with stout quadrangular winged stems, oval leaves, jalap-like white tuberous roots, clusters of tubular-campanulate white flowers and round fruits, grows in almost all areas of Bangladesh (Ghani 2003). It is used as medicinal plants (Austin 1982). Turpeth root is chiefly used in small to moderate doses to clear bowels (Chevallier 1996).

13.4 Objectives

Thus the aim of the present study is to develop environment friendly and economically viable protocol by using local herbal products/plants against fish and shrimp diseases.

13.5 Specific Objectives

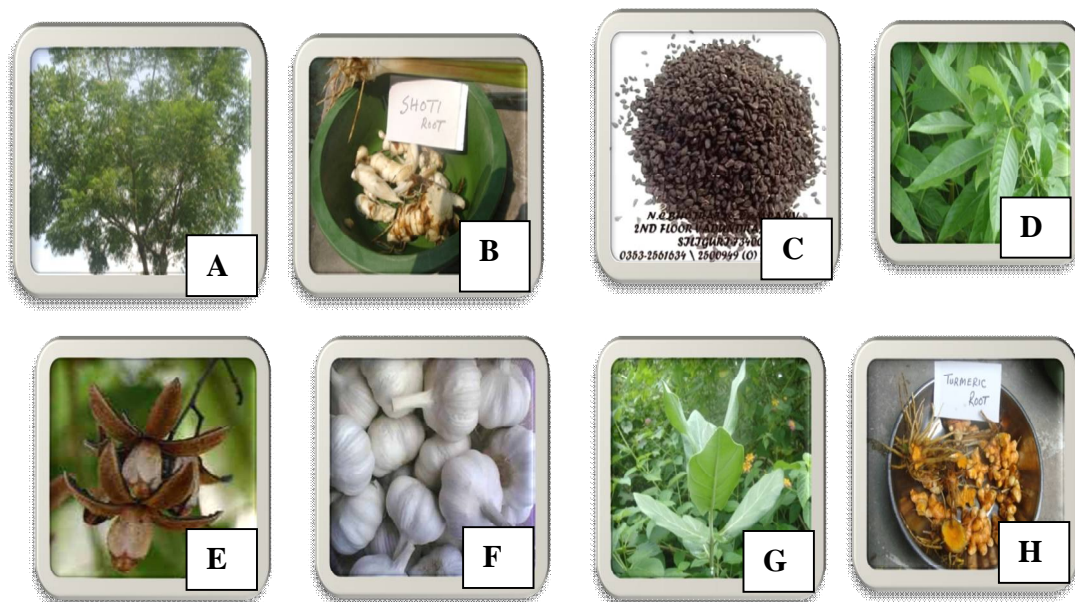
- i. Survey and selection of potential medicinal plants/products against bacterial, fungal and parasitic diseases.

- ii. Collection of crude and fine extract from herbal products.
- iii. Use of extract as feed supplement and in bath treatment in aquarium and pond against diseases.
- iv. Isolation of bacteria, fungus, and parasites for disease identification.
- v. Use of histopathology to know disease recovery.

13.6 Experimental design and Methodology

Survey and selection of medicinal plants

Medicinal plants and products of Bangladesh were surveyed following scientific works, reviews and internet search (Table 69). Plants and plant products having antibacterial, antifungal and ant parasitic properties were given preference during survey and selection. Medicinal plants were selected on the basis of availability, social acceptability, habitat and review of literature (Table 70). Selected medicinal plants were Neem leaf, seed, Kalojira seed, Mahagoni seed, Garlic bulb, Turmeric root, Shoti rhizome, Akand leaf, Basok leaf, Arjun bark and Amrul leaf (Figs 32A-32H).



Figs. 32 A, Neem leaf, B, Shoti rhizome, C, Kalojira, D, Basak leaf, E, Mahagoni seed, F, Onion, G, Akand leaf and H, Turmeric rhizome

Table 69 Local and scientific name of medicinal plants in Bangladesh and their uses

SL No	Local name	Scientific name	Family	Using part	Control Disease name
1.	Apang	<i>Achyranthes Paniculata</i>	Amaranthaceae	whole plant	Dysentery, Constipation, piles, Arthritis, Skin disease.
2.	কালোমেঘ	<i>Andrographis pariculata</i>	Acanthaceae	whole plant	Metabolic problem, Gastric, Fever, worm killer, Dysentery, Liver Disease, Strengthen.
3.	আকন্দ	<i>Calotropis procera</i>	Asclepiadaceae	Root, leaf. Bark flower extract of leaf.	Ulcer, Tooth pain chronic dysentery, cold, Asthma,
4.	অনন্তমূল	<i>Hemidesmus indicus</i>	Asclepiadaceae	Root and whole plant	Strength increaser, apetiser. Arthritis, Diabetes.
5.	অর্জুন	<i>Terminalia arjuna</i>	Combretaceae	Bark	Heart disease, Diarrhea, piles, Tuberculosis.
6.	উলটকম্বল	<i>Abroma augusta</i>	Sterculiaceae	Root, Bark & Leaf	Vaginal pain sexual disease.
7.	ঘৃতকুমারী	<i>Aloe indica</i>	Liliaceae	Extract of leaf	Headache, sexual disease, metabolic problem. Fever.
8.	খানকুনি	<i>Centella asiatica</i>	Apiaceae	Whole plant	Metabolic problem, pain killer Diabetics, Ulcer, chronic dysentery, Anti Coughing.
9.	তেলাকুচা	<i>Eoccinia cordifolia</i>	Cucarbitace	Leaf and root	Diabetics, cold, appetizer, Ulcer control etc.
10.	কেশরাজ	<i>Eclipta Prostrata</i>	Asteraceae	Whole plant	Headache cold, control of hair losing
11.	Kulakhara	<i>Hygrophilla schulli</i>	Acanthaceac	Leaf, Seed, Steam	Control of anger, Liver disease, Ulcer, bleeding, removal of stone from kidney.
12.	Gondho vadule.	<i>Paedaria foetida</i>	Rubiaceae	Leaf	Dysentery, metabolic disorder, cold. Arthritis.
13.	ধুতরা	<i>Datura metal</i>	Solanaceac	Root, Leaf, Seed	Pain killer worn killer, poisonous
14.	বহেরা	<i>Terminalia belerica</i>	Combreta ceae	Fruit	Constipation, Diarrhea, fever, cough, piles, Gastric, Heart disease.

SL No	Local name	Scientific name	Family	Using part	Control name	Disease
15.	দাদমর্দন	<i>Cassia alata</i>	Fabaceae	Leaf	Skin	disease, poisonous.
16.	পুদিনা	<i>Mentha viridis</i>	Lebiatae	Whole plant	Metabolic	disorder, Gastric.
17.	বাসক	<i>Adhatoda vasica</i>	Acanthaceae	Leaf, root of plant	Cough,	asthma, tuberculosis, cold, blood refine.
18.	জ্যোষ্ঠিমধু	<i>Hydrangea arborescons</i>	Saxifra zaceac	Leaf flower fruit	Liver disease,	adrenal peptic Ulcer, hormonal disease, cold, throat pain.
19.	ব্রাহ্মীশাক	<i>Becopa moniera</i>	Scrophulariaceae	Leaf	Heart disease,	nurval pressure, Asthma.
20.	শর্পগন্ধা	<i>Rauwolfia Serpentina</i>	Apocynaceae	Leaf and root.	Blood Pressure,	brain abnormal, dysentery diarrhea pain killer.
21.	কুরচি	<i>Holarrhena antidysenterica</i>	Apocynaceae	Bark & Seed	Diarrhea,	dysentery, worm killer constipation, intestinal weakness.
22.	নিম	<i>Azadirachta indica</i>	Meliaceae	Root, leaf Bark	Skin disease,	worm killer Arthritis, Insecticide, Anti vomiting, Tooth disease, Jaundice etc. Antiviral.
23.	উলটচণ্ডাল	<i>Gloriosa superba</i>	Liliaceae	Leaf and Steam	Arthritis	Adrenals peptic, ulcer.
24.	শতমূলী	<i>Asparagus racemosus</i>	Liliaceae	Leaf and root	Fever	dysentery Diabetics.
25.	বেল	<i>Aegle marmelos</i>	Rutaceae	Fruit	Dysentery	Diarrhoea.
26.	আনারস	<i>Ananas comosus</i>	Bromeliaceae	Fruit & leaf	Jaundice	
27.	জাম	<i>Syzygium cumini</i>	Myrtaceae	Fruit & seed	Diabetics,	Dysentery.
28.	আসামলতা	<i>Mikania scandens</i>	Compositae	Leaf	Bleeding control,	Dysentery, Daud etc.
29.	নিশিন্দা	<i>Vitex negundo</i>	Verbenaceae	root. leaf	Asthma,	Arthritis, fever etc.
30.	দুর্বা	<i>Cynodon dactylon</i>	Grominae	Leaf	Blood	bleeding control. skin disease.