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Antibacterial and Antifungal Activities of Essential Oils Extracted from Medicinal Plants Using CO_2 Supercritical Fluid Extraction Technology

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Antibacterial and antifungal activities of 110 medicinal plants most commonly used in Pakistani medicine were determined by zone inhibition method. The essential oils from medicinal plants were extracted using CO_2 supercritical fluid extraction technology. The oils were subsequently purified under reduced pressure to avoid degradation of their components. Strongest *Bacillus subtilis* inhibition was shown by *Petrocarpus santalinus, Valeriana officinalis, Caryophyllus arometicus, Trapa bispinosa* and *Mentha arrivensis*. Whereas, strongest *Ganoderma luciderm* inhibition activity was shown by *Mentha arrivensis, Hibiscus esulentus* and *Acacia concinna*. Many medicinal plants have not shown any inhibition activity against *Bacillus subtilis* and *Ganoderma lucidum*. Results of the present study can be very helpful in evaluating the mechanism of action of essential oil extracted from medicinal plants using CO_2 supercritical fluid extraction technology.

Key Words: Antibacterial, Antifungal, Essentials oils, Medicinal plants.

INTRODUCTION

Many foods are deteriorated as a result of microbial activity. These spoilages may out break into food borne diseases[1]. Essential oils from medicinal plants can be used for microbial resistance development in food items[2,3]. The use of natural preservatives should be preferred due to carcinogenic and teratogenic attributes as well as residual toxicity of chemical preservatives[4,5]. Essential oils are terpene hydrocarbons as well as their oxygenated derivatives such as alcohols, aldehydes, ketones, acids and esters. Essential oils are extracted from different plants parts[6]. Essential oils perform various functions in plants including conferring pest and disease resistance and are known to possess antifungal, acaricidal, antibacterial and cytotoxic activities[7-15]. Therefore, they are screened to apply in the fields of pharmacology, pharmaceutical botany, medicinal and clinical microbiology, phytopathology and food preservation[16].

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In this regard, the present study is undertaken to screen antifungal and antibacterial ability of essential oils extracted from indigenous medicinal plants using CO_2 supercritical fluid extraction technology.

EXPERIMENTAL

Essential oil extraction: Five kilograms of 110 dried medicinal plants (Table-1) were purchased from Herbal Medicinal Plant Store, Faisalabad, Pakistan. The essential oil from these medicinal plants was extracted using CO_2 supercritical fluid extraction technology. The essential oils yields are given in Table-1. The CO_2 tank, extractor and separator pressures were maintained at 50, 90 and 50 bars, respectively. Temperatures of CO_2 tank, extractor and separator were maintained at 5, 40 and 35 °C, respectively. The isolated essential oils were further purified under reduced pressure using a rotary evaporator. Finally, essentials were dried over anhydrous sodium sulphate and kept at 4 °C for other experiments.

Chemicals and microorganisms: All chemicals were of high purity grade and were purchased from Fluka Chemicals. *Bacillus subtilis* and *Ganoderma lucidum* cultures were obtained from Molecular Biology/Industrial Biotechnology Laboratory, Department of Chemistry and Biochemistry, University of Agriculture, Faisalabad, Pakistan.

Determination of antifungal and antibacterial activity: *Ganoderma lucidum* was grown on potato dextrose agar (PDA) medium for sporulation on petri dishes for 7 days[17]. Whereas *Bacillus subtilis* was cultured overnight at 37 °C in Mueller Hinton Agar (MHA) medium. The antifungal and antibacterial activities of essential oils were determined by measuring the size of zone of inhibition. Filter papers having a diameter of 6 mm, soaked with 20 µL of essential oil were placed in petri plates. Before incubation, all petri plates were placed at 4 °C for 2 h in a cooled cabinet. Then these plates were incubated at 37 °C for 24 h for *Bacillus subtilis* and at 30 °C for 48 h for *Ganoderma lucidum*. The diameters of the inhibition zones were measured in millimeters[18]. All samples were studied in triplicate to obtain reproducible results.

RESULTS AND DISCUSSION

The obtained results clearly demonstrated that various essential oils exhibited varying levels of antimicrobial activities. Some essential oils have not shown any antimicrobial activity. *Peterocarpus santalinus, Valeriana officinalis, Caryophyllus arometicus* linn., *Trapa bispinosa* Roxb. and *Mentha arvensis* have shown strongest *Bacillus subtilis* activities (Table-2). Valeriana officinalis has shown the strongest antibacterial activity. *Mentha arvensis* was most prominent in its antifungal activity. *Hibiscus esculentus* and *Acacia concinna* also showed strongest activities against *Ganoderma lucidum*. In most of cases essential oil extracted from 95 medicinal plants have shown moderate antibacterial activity and no antifungal activity. The basis of varying degree of sensitivity of test organisms may be due to the intrinsic

TABLE-1
MEDICINAL PLANTS USED IN THE PRESENT STUDY

Sr. No.	Local name	Common name	Botanical name	Family name	Part used	Oil yield %
1	Ajneer	Fig	*Ficus carica* L.	Moraceae	Fruit	5.32
2	Neem	Neem	*Azadirachta indica* L.	Meliaceae	Bark	8.28
3	Jaiphal	Nutmeg	*Myristica fragran houtt*	Myristicaceae	Seed kernels	5.93
4	Kali musli	Musali	*Curculigo orchioides Gaertn.*	Amaryllidaceae	Stalk	6.72
5	Revandhinu	Rhu barb	*Rheum emodi wall*	Polygonaceae	Leaves	8.57
6	Chandan	Sandal wood	*Santalum Album* L.	Santalaceae	Wood	6.46
7	Choob cheeni	Choob cheeni	*Smilax ovalifolia* Roxb.	Smilacaceae	Root	5.66
8	Rai	Black mustard	*Brassica nigra* L.	Cruciferae	Seed	5.35
9	Mulethi	Liquorice	*Glycyrrhiza glabra* L.	leguminosae	Root	5.17
10	Catjang	Jerusalem pea	*Vigna unguiculata (l.) walp*	Leguminosae	Seed	4.34
11	Lavender	Common lavender	*Lavandula angustifolia*	Lamiaceae	Flowers	4.25
12	Mosli safaid	Mosli safaid	*Chlorophutum arunddinaceum bake*	Liliaceae	Stalk	5.06
13	Khitmi	Marshmallow	*Althaea officinalis*	Malvaceae	Root	5.83
14	Sarsoon	Red mustard	*Brassica juncea*	Brassicaceae	Seed	5.09
15	Muli	Radish	*Raphanus sativus* L.	Curciferae	Root	5.07
16	Khaskhash	Poppy seeds	*Papaver somnifrum* L.	Papaveraceae	Seed	3.62
17	Balaikand	Vidarikand	*Ipomoea digitata*	Convolvulaceae	Root	3.70
18		kangi	*Euphorbia dracunculoides lam*	Euphorbiaceae	Seed	4.61
19	Badian-e-khatai	Star anise	*Illicium Verum Hook.*	Magnoliaceae	Fruit	6.2
20	Chiraita	Chiraita	*Swertia chirata buch ham*	Gentianaceae	Branch	5.84
21	Sumander sokh	Elephant Creeper	*Argyreia speciosa Sweet*	convolvulaceae	Root	4.02
22	Kachur	Zedoary	*Curcuma zedoaria*	Zingiberaceae	Dried rhizomes	4.49
23	Kikar	Babul	*Acacia Arabica wild*	Leguminosae	Poods	4.56
24	Aganvood	Aganvood	*Aquilaria agallocha roxb.*	Thymelaeaceae	Wood	4.68
25	Brahmi booti	Jal Brahmi	*Centella asiatica*	Umbelliferae	Leaves	4.60
26		Majithlall	*Symplocos racemosa roxb.*	Styraceae	Bark	5.57

27		Yarrow	*Achillea millefolium* L.	Asteraceae	Flowers	6.24
28		Kantakri	*Solanum surattense burm F.*	Solanaceae	Whole plant	4.65
29	Guncai	Wild liquorice	*Abrus precatorius* L.	Papilionaceae	Leaves	3.91
30	Compass Plant	Wild lettuce	*Lactuca serriola* L.	Compositae	Leaves	5.26
31	Banafsa	(Sweet Violet)	*Viola odorata* L.	Violaceae	Leaves	7.87
32	Ratanjot	Alkanet root	*Onosma echioidea* L.	Boraginaceae	Leaves	6.45
33	Til	Sesame	*Sesamum indicum* L.	Arecaceae	Fruit	5.10
34	Kikar	Babol	*Accassia Arabica wild*	Papilionaceae	Bark	4.86
35	Lady finger	Okra	*Hibiscus esulentus* L.	Malvaceae	Seed	5.68
36	Kasni	Chicory	*Cichorium intybus* L.	Compositae	Leaf	7.43
37	Kalonji	Black caraway seed or black cumin	*Nigella sativa* L.	Ranunculaceae	Seed	5.05
38	Ajmud	Celery	*Antheum graveolense* L.	Apiaceae	Fruit	6.26
39	Anwala	Embolic Myrobalan, Indiana gooseberry	*Embolica officinalis gaertn*	Euphorbiaceae	Fruit	7.25
40	Arand	Castor	*Ricinus communis* L.	Euphorbiaceae	Seed	10.02
41	Hing	Asafoetida	*Ferula foetida regel*	umbelliferae	Resin	8.26
42	Dhak	Bastard teak	*Butea frondosa roxb.*	Fabaceae	Seeds	6.54
43	Soom	Ephedra	*Ephedra gerardiana.* Wall	Gnetaceae	Whole plant	7.6
44	Kali zeerli	Black/bitter cumin	*Centratherum anthelminticum* L.	Asteraceae	Seeds	7.33
45		Canadian milkvetch	*Astragalus carolinianus*	Leguminosae	Root	6.67
46	Alubokhara	Commom plum	*Prunus domestica* L.	Rosaceae	Dried Fruit	8.53
47	Ginseng	Oriental ginseng	*Panax notoginseng*	Araliaceae	Fruit	8.02
48	Pippali	Long Pepper	*Piper longum* L.	Piperaceae	Dried spikes	8.34
49	Red sandal	Red sandal wood	*Petrocarpus santalinus*	Fabaceae	Stem bark	7.76
50		Pennyroyal	*Mentha pulegium* L.	Labiatae	Leaves	5.37
51	Kikar	Kikar	*Acacia Arabica(Lam.) Wild*	Papilionaceae	Bark	5.05
52	Daarunaj Aqrabi	Leopard's Bane	*Doronicum hookeri hook*	Compositae	Root	5.45
53	Underjosheren	Dyers's Oleander	*Wrightia Tinctoria (roxb.) R. Br.*	Apocynaceae	Bark	7.26

54	Amba haldi		*Curcuma amada Roxb.*	Zingiberaceae	Rhizomes	6.38
55	Kababchini	Cubebs	*Piper cubeba* L.	Piperaceae	Fruit	4.92
56	Tulasi	Sacred basil	*Ocumum sanctum* L.	labiatae	Seed	5.62
57	Wax tree	Redlac sumac	*Rhus succedanea* L.	Anacardiaceae	Fruit	5.20
58	Khabazi	Mallow	*malva sylvestris* L.	Malvaceae	Leaves	5.21
59	Sont	Ginger	*Zingiber montanum*	Zingiberaceae	Rhizome	5.59
60	Kapoor	Camphor	*Cinnamomum camphora nees & Eberm.*	lauraceae	Leaves	3.48
61	Mehandi	Henna	*Lawsonia inermis* L.	Lythraceae	Leaves	7.16
62	Dalchini	Cinnamon	*Cinamomum zeylanicum blume*	lauraceae	Bark	6.08
63		Shyonaka	*Oroxylum indicum vent.*	Bignoniaceae	Bark	7.34
64	Haloon, haraf-haleem	Chinese motherwort	*Lepidium sativum* L.	Lamiaceal	Fruit	5.37
65	Aspaghol	Hoary plantain	*Plantago ovata forssk*	Plataginaceae	Fruit	7.36
66	Babachi	Sole, sole berries	*Psorolea corylifolia* L.	Papillionaceae	Fruit	5.98
67	Ulsi	Linseed	*Linum usitatissimum* L.	linaceae	Seed	5.12
68	Methi	Red clover	*Trigonella foencum-graecum* L.	Fabaceae	Leaf	8.01
69	Zira sufaid	Cumin	*Cuminum cyminum* L.	umbelliferae	Fruit	6.03
70		Chitraka	*Plumbago zeylancia* L.	Plumbaginaceae	Root	7.76
71			*Amomum subulatum* Roxb.	Zingiberaceae	Seed	8.69
72		Sarpagandha	*Rauwolfia serpentine benth.*	Apocynaceae	Root	8.07
73	Lalmirch	Red pepper	*Capsicum annuum*	Solanaceae	Fruit	7.76
74	Tejpat	Cinnamon leaf	*Cinnamon tamala nees & eberumx*	Lauraceae	Leaf	6.31
75	Haldi	Turmeric	*Curcuma longa*	Zingiberaceae	Root	3.56
76	Saunf	Fennel	*Foeniculum vulgare Mill.*	Umbelliferae	Fruit	5.56
77	Kali mirch or golmirichi	Black pepper	*Piper nigrum* L.	Piperraceae	Fruit	4.52
78	Chestnut	Sweet Chestnut	*Castanea sativa Mill.*	Fagaceae	Bark	4.29
79		Vasaka	*Adhatoda vasica nees.*	Acanthaceae	Roots Roots	6.63
80	Tea	Tea	*Camellia thea*	Camelliaceae	Dried leaf	5.14
81	Chareela	Rock lichens	*Parmelia perlata ach*	Parmeliaceae	Branches	0.67

82	Sahtere	Fumitory	*Fumaria officinalis* L.	Fumariaceae	Branches	7.43
83	Unab	Jujube	*Zizyphus jujube*	Rhamanaceae	Fruit	8.62
84	Asgand	Withania Aswagandh	*Withania somnifera (L.)Dunal*	Solanaceae	Root	4.94
85		Knotweed	*Polygonum aviculare*	Polygonaceae	Whole plant	8.43
86	Asshokh kanoja	American mistletoe	*Phyllanthus maderaspatensis* L.	Viscaceae	Fruit	5.86
87	Baberang	Vidanga	*Embelia ribes Burm*	Myrsinaceae	Dried Fruit	4.75
88		Methi	*Trigonella foenumgraecum* L.	Papilionaceae	Leaves, Seed	4.28
89		Devil's Cotton	*Abroma augusta*	Sterculiaceae	Leaves	3.92
90	Sumach	Dyer's sumac	*Rhus coriaria*	Anacardiaceae	Fruit	4.76
91	Harara	Chebulic myrobalan	*Terminalia chebula Retz.*	Combretaceae	Fruit	5.12
92		Shekakai	*Acacia concinna Dc*	Mimosaceae	Fruit	3.47
93		Valerian	*Valeriana officinalis*	Valerianaceae	Root	3.93
94	Charungi	Nigaki	*Picrasma quassioides benett*	Simaroubaceae	Bark	6.71
95	Loung	Cloves	*Caryophyllus arometicus L.*	Myrtaceae	Fruit	4.98
96		Wallflower	*Erysimum cheiri (L.) Crantz.*	Cruciferae	Branches	3.67
97	Bala	Country Mallow	*Sida cordifolia* L.	Malvaceae	Root,	3.82
98	Tabasheer	Bamboo	*Bambusa arundinaceae wild*	Gramineae	Leaves	4.58
99	Daniya	Coriander	*Coriandrum sativum* L.	Apiaceae	Whole plant	4.65
100		Autumn Crocus	*Colchicum autumnale* L.	Colchicaceae	Seeds	4.85
101	Ajvain	Caraway	*Carum copticum* L.	Asteraceae	Fruit	5.12
102	Avellano	Hazel	*Coryus avellana* L.	Betulaceae	Bark	4.62
103	Tarbut	Indian jalap	*Operculina turpethum*		Bark	6.23
104	Aoud saleeb	Mamekh	*Paeonia emodi* Wall.	Paeoniaceae	Root	6.76
105	Jou	Barley	*Hordeum vulgare subsp. Vulgare*	Poaceae	Fruit	6.52
106	Manjith	Indian Madder	*Rubia cordifolia* L.	Rubiaceae	Root	5.78
107	Bahera	Beleric myrobalan	*Terminalia belerica*	Combretaceae	Fruit	5.96
108	Karonja	Galangal	*Alpinia galangal* L.	Zingiberaceae	Rhizome	6.22
109	Singhara	Water chest nut	*Trapa bispinosa rex* B.	onagraceae	Fruit	5.67
110	Japnese mint	Corn Mint	*Mentha arvensis* L.	Lamiaceae	Whole plant	11.8

TABLE-2
ANTI *Bacillus subtilis* ACTIVITY OF MEDICINAL PLANTS ESSENTIAL OILS

Sr. No.	Scientific name	Zone of inhibition (mm)
1	*Ficus carica* L.	14
2	*Azadirachta indica* L.	11
3	*Myristica fragran houtt*	20
4	*Curculigo orchioides Gaertn.*	12
5	*Rheum emodi wall*	16
6	*Santalum Album* L.	Not shown
7	*Smilax ovalifolia* Roxb.	22
8	*Brassica nigra* L.	10
9	*Glycyrrhiza glabra* L.	17
10	*Vigna unguiculata (l.) walp*	12
11	*Lavandula angustifolia*	22
12	*Chlorophutum arunddinaceum bake*	11
13	*Althaea officinalis*	20
14	*Brassica juncea*	18
15	*Raphanus sativus* L.	12
16	*Papaver somnifrum* L.	24
17	*Ipomoea digitata*	Not Shown
18	*Euphorbia dracunculoides lam*	12
19	*Illicium Verum Hook.*	14
20	*Swertia chirata buch ham*	14
21	*Argyreia speciosa Sweet*	Not Shown
22	*Curcuma zedoaria*	16
23	*Acacia Arabica wild*	15
24	*Aquilaria agallocha roxb.*	Not Shown
25	*Centella asiatica*	18
26	*Symplocos racemosa roxb.*	12
27	*Achillea millefolium* L.	18
28	*Solanum surattense burm F.*	Negligible
29	*Abrus precatorius* L.	Not shown
30	*Lactuca serriola* L.	16
31	*Viola odorata* L.	18
32	*Onosma echioidea* L.	22
33	*Sesamum indicum* L.	Not Shown
34	*Accassia Arabica wild*	20
35	*Hibiscus esulentus* L.	36
36	*Cichorium intybus* L.	11
37	*Nigella sativa* L.	Not Shown
38	*Antheum graveolense* L.	12
39	*Embolica officinalis gaertn*	25
40	*Ricinus communis* L.	20
41	*Ferula foetida regel*	Not shown
42	*Butea frondosa roxb.*	Negligible
43	*Ephedra gerardiana.* Wall	Not Shown
44	*Centratherum anthelminticum* L.	12
45	*Astragalus carolinianus*	Not Shown

46	*Prunus domestica* L.	Not Shown
47	*Panax notoginseng*	18
48	*Piper longum* L.	Not Shown
49	*Petrocarpus santalinus*	30
50	*Mentha pulegium* L.	11
51	*Acacia Arabica(Lam.) Wild*	Not Shown
52	*Doronicum hookeri hook*	Not Shown
53	*Wrightia Tinctoria (roxb.) R. Br.*	15
54	*Curcuma amada Roxb.*	19
55	*Piper cubeba* L.	17
56	*Ocumum sanctum* L.	13
57	*Rhus succedanea* L.	Negligible
58	*malva sylvestris* L.	12
59	*Zingiber montanum*	12
60	*Cinnamomum camphora nees & Eberm.*	22
61	*Lawsonia inermis* L.	15
62	*Cinamomum zeylanicum blume*	14
63	*Oroxylum indicum vent.*	24
64	*Lepidium sativum* L.	12
65	*Plantago ovata forssk*	Negligible
66	*Psorolea corylifolia* L.	12
67	*Linum usitatissimum* L.	14
68	*Trigonella foencum-graecum* L.	20
69	*Cuminum cyminum* L.	22
70	*Plumbago zeylancia* L.	12
71	*Amomum subulatum* Roxb.	14
72	*Rauwolfia serpentine benth.*	20
73	*Capsicum annuum*	12
74	*Cinnamon tamala nees & eberumx*	22
75	*Curcuma longa*	20
76	*Foeniculum vulgare Mill.*	Negligible
77	*Piper nigrum* L.	16
78	*Castanea sativa Mill.*	13
79	*Adhatoda vasica nees.*	Not shown
80	*Camellia thea*	16
81	*Parmelia perlata ach*	24
82	*Fumaria officinalis* L.	26
83	*Zizyphus jujube*	12
84	*Withania somnifera (L.)Dunal*	Not Shown
85	*Polygonum aviculare*	Not Shown
86	*Phyllanthus maderaspatensis* L.	14
87	*Embelia ribes Burm*	Not Shown
88	*Trigonella foenumgraecum* L.	10
89	*Abroma augusta*	12
90	*Rhus coriaria*	11

91	*Terminalia chebula Retz.*	13
92	*Acacia concinna Dc*	18
93	*Valeriana officinalis*	40
94	*Picrasma quassioides benett*	12
95	*Caryophyllus arometicus L.*	33
96	*Erysimum cheiri (L.) Crantz.*	Not Shown
97	*Sida cordifolia* L.	Not shown
98	*Bambusa arundinaceae wild*	14
99	*Coriandrum sativum* L.	10
100	*Colchicum autumnale* L.	Not Shown
101	*Carum copticum* L.	18
102	*Coryus avellana* L.	22
103	*Operculina turpethum*	16
104	*Paeonia emodi* Wall	Not Shown
105	*Hordeum vulgare subsp. vulgare*	23
106	*Rubia cordifolia* L.	20
107	*Terminalia belerica*	17
108	*Alpinia galangal* L.	12
109	*Trapa bispinosa rex* B.	30
110	*Mentha arvensis* L.	32

tolerance of microorganisms and the nature and combinations of phyto-compounds present in the essential oil[18]. The volatile essential oils consist of complex mixture of numerous components. Thee major or trace compound(s) might give rise to antimicrobial activity. However, some authors think that essential oil activity should be considered as whole[19]. The chemical components of essential oil exert their toxic effects, against studied microorganisms through the disruption of bacterial or fungal integrity.

Trapa bispinosa Roxb is an aquatic floating herb as a folkloric reputation as a cure for diarrhea, dysenteriae, aphrodisiae, astringent to the bowels, leprosy, inflammations, urinary discharges, fractures, sore throat, bronchitis, leucorrhoea, bad teeth and malaria[20]. Rehman *et al.*[20] reported that *Trapa bispinosa* Roxb acts as a strong inhibitor of *Bacillus subtilis*. *Mentha arvensis* is valued especially for its antiseptical property and its beneficial effect on the digestion. *Mentha arvensis* is the only plant in the present study which has shown strongest antibacterial and antifungal properties (Table-2). *Caryophyllus arometicus* linn. has benefit in cases of nervous tension, depression, irritability, hysteria, panic, anxiety fear, stomach cramping, indigestion due to nervousness, delusions and exhacestion. *Peterocarpus santalinus* is used as astringent, diaphoretic, inflammations, headache, skin disease, bilious infections and chronic dysentery[21]. *Hibiscus esculentus* L. commonly known as lady finger. The essential oil extracted from its flower has shown strong antigenoderma lucidum activity (Table-3). *Acacia concinna* is used against dyspepsia, hepatitis, jaundice, adiaphoresis, dysentery, insomnia, loss of concentration, indigestion, skin infection and insect bite.

TABLE-3
ANTIFUNGAL ACTIVITIES OF MEDICINAL PLANTS
ESSENTIAL OILS AGAINST *Ganoderma lucidum*

Sr. No.	Scientific name	Zone of inhibation (mm)
1	*Ficus carica* L.	10
2	*Azadirachta indica* L.	28
3	*Myristica fragran houtt*	18
4	*Curculigo orchioides Gaertn.*	Negligible
5	*Rheum emodi wall*	13
6	*Santalum Album* L.	Not Shown
7	*Smilax ovalifolia* Roxb.	14
8	*Brassica nigra* L.	Not Shown
9	*Glycyrrhiza glabra* L.	12
10	*Vigna unguiculata (l.) walp*	16
11	*Lavandula angustifolia*	22
12	*Chlorophutum arunddinaceum bake*	Negligible
13	*Althaea officinalis*	18
14	*Brassica juncea*	Not Shown
15	*Raphanus sativus* L.	16
16	*Papaver somnifrum* L.	20
17	*Ipomoea digitata*	11
18	*Euphorbia dracunculoides lam*	15
19	*Illicium Verum Hook.*	18
20	*Swertia chirata buch ham*	22
21	*Argyreia speciosa Sweet*	10
22	*Curcuma zedoaria*	24
23	*Acacia Arabica wild*	28
24	*Aquilaria agallocha roxb.*	14
25	*Centella asiatica*	11
26	*Symplocos racemosa roxb.*	20
27	*Achillea millefolium* L.	12
28	*Solanum surattense burm F.*	Negligible
29	*Abrus precatorius* L.	15
30	*Lactuca serriola* L.	Not Shown
31	*Viola odorata* L.	25
32	*Onosma echioidea* L.	36
33	*Sesamum indicum* L.	26
34	*Accassia Arabica wild*	30
35	*Hibiscus esulentus* L.	30
36	*Cichorium intybus* L.	16
37	*Nigella sativa* L.	24
38	*Antheum graveolense* L.	15
39	*Embolica officinalis gaertn*	20
40	*Ricinus communis* L.	12
41	*Ferula foetida regel*	Negligible
42	*Butea frondosa roxb.*	13
43	*Ephedra gerardiana.* Wall	12
44	*Centratherum anthelminticum* L.	12

45	*Astragalus carolinianus*	Not Shown
46	*Prunus domestica* L.	Not Shown
47	*Panax notoginseng*	14
48	*Piper longum* L.	15
49	*Petrocarpus santalinus*	Negligible
50	*Mentha pulegium* L.	12
51	*Acacia Arabica(Lam.) Wild*	Not Shown
52	*Doronicum hookeri hook*	Not Shown
53	*Wrightia Tinctoria (roxb.) R. Br.*	Negligible
54	*Curcuma amada Roxb.*	Negligible
55	*Piper cubeba* L.	10
56	*Ocumum sanctum* L.	Not Shown
57	*Rhus succedanea* L.	Not shown
58	*malva sylvestris* L.	Not Shown
59	*Zingiber montanum*	Not shown
60	*Cinnamomum camphora nees & Eberm.*	25
61	*Lawsonia inermis* L.	Negligible
62	*Cinamomum zeylanicum blume*	12
63	*Oroxylum indicum vent.*	14
64	*Lepidium sativum* L.	13
65	*Plantago ovata forssk*	Not Shown
66	*Psorolea corylifolia* L.	15
67	*Linum usitatissimum* L.	Negligible
68	*Trigonella foencum-graecum* L.	12
69	*Cuminum cyminum* L.	Negligible
70	*Plumbago zeylancia* L.	Not Shown
71	*Amomum subulatum* Roxb.	15
72	*Rauwolfia serpentine benth.*	11
73	*Capsicum annuum*	11
74	*Cinnamon tamala nees & eberumx*	16
75	*Curcuma longa*	18
76	*Foeniculum vulgare Mill.*	Negligible
77	*Piper nigrum* L.	12
78	*Castanea sativa Mill.*	15
79	*Adhatoda vasica nees.*	Not Shown
80	*Camellia thea*	13
81	*Parmelia perlata ach*	20
82	*Fumaria officinalis* L.	11
83	*Zizyphus jujube*	10
84	*Withania somnifera (L.)Dunal*	18
85	*Polygonum aviculare*	Negligible
86	*Phyllanthus maderaspatensis* L.	10
87	*Embelia ribes Burm*	Not Shown
88	*Trigonella foenumgraecum* L.	Not Shown
89	*Abroma augusta*	Negligible
90	*Rhus coriaria*	11
91	*Terminalia chebula Retz.*	Not shown
92	*Acacia concinna Dc*	30

93	*Valeriana officinalis*	Not Shown
94	*Picrasma quassioides benett*	12
95	*Caryophyllus arometicus L.*	25
96	*Erysimum cheiri (L.) Crantz.*	10
97	*Sida cordifolia* L.	Not shown
98	*Bambusa arundinaceae wild*	13
99	*Coriandrum sativum* L.	15
100	*Colchicum autumnale* L.	Not Shown
101	*Carum copticum* L.	20
102	*Coryus avellana* L.	16
103	*Operculina turpethum*	11
104	*Paeonia emodi* Wall.	Not Shown
105	*Hordeum vulgare subsp. vulgare*	Negligible
106	*Rubia cordifolia* L.	14
107	*Terminalia belerica*	Not shown
108	*Alpinia galangal* L.	Negligible
109	*Trapa bispinosa rex* B.	16
110	*Mentha arvensis* L.	60

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