Chapter II

Review of Literature

I. Antibiotics from higher plants

The search for new antibiotics has been most intensive among the lower plants, for various reasons, with special emphasis on various *Streptomycetes* and a few fungi. Certain disease entities, however, remain causing serious problems and some of the major antibiotics have considerable drawbacks in terms of limited antimicrobial spectrum or serious side effects. These factors impel a continuing search for new agents. Especially needed are safe antibiotics effective against clinical infections caused by gram-negative organisms, fungi, viruses and mycobacteria.

It is reasonable to suppose that clinical and commercially significant new antibiotics with activities supplemental to and structures widely different from those in current use might be found in sources which have not as yet been as thoroughly explored as the traditional microorganisms. Reports are appearing in the antibiotic literature with increasing frequency describing new antibiotics from such microbial sources as the *Micromonospora*, *Nocardia*, *Microbispora*, certain fungi, etc. However, the search for new antibiotics is no longer restricted primarily to microbial products. Recently, constituents of higher plants have exhibited significant antimicrobial activity, as listed in Table 1.

Table 1 Antimicrobial activity from higher plants

Botanical origin	Part(s) used	Antimicrobial compound(s)	Biological activity	Ethnobotanic use	Reference
Gymnosperms					
Cupressaceae					
Juniperus sabina L. and other spp.	Leaves	Podophyllotoxin	Antitumor	-	Lewis and Elvin-lewis, 1977
Thuja plicata Donn	Wood	Tetrahydroxy-stillbene, Thujaplicins	Antifungal (cellulose-decomposing fungi)	-	
Ginkgoaceae					
Ginkgo biloba L.	Fruit	Ginkgolic acid	Antibacterial (Tubercle bacilli)	-	
Pinaceae					
Pinus sylvestris L.	Heartwood	Pinosylvine	Antibacterial, antifungal	-	
Angiosperms					
Anacardiaceae					
Anacardium occidentale L.	Oil from fruit	Anacardic acid	Antibacterial (gram positive bacteria), anthelmintic	Against leprosy and anthelmintic	
Apocynaceae					
Rhazya stricta Decsne.	Leaves	Stemmadenine	Antibacterial (*S. aureus*, *E. coli*, *Ps. aeruginosa*)	Sore throat, syphilis, fever, cancer and general debility	Mariee et al., 1988

Table 1 (cont.)

Botanical origin	Part(s) used	Antimicrobial compound(s)	Biological activity	Ethnobotanic use	Reference
Aristolochiaceae					
Aristolochia clematitis L.	-	Substances A and B	Antibacterial (broad spectrum), antifungal	For cold, chills, fever asthma	Lewis and Elvin-lewis, 1977
Asarum canadense L., *A. europaeum*	Root	Substances A and B	Antibacterial, antifungal	Treating coughs	
Balsaminaceae					
Impatiens balsamina L.	Plant	2-Methoxy-naphthoquinone	Antifungal	-	
Berberidaceae					
Berberis vulgaris L.	Bark of stems and roots	Berberine	Antibacterial (broad spectrum), antiprotozoal	-	
Brassicaceae					
Brassica alba (L.) Rabenh., *B. juncea* (L.) Czern.et Coss., and *B. nigra* (L.) Koch.	Dried ripe seeds	Volatile mustard oil (allyl isothiocyanate)	Antibacterial, antifungal	Appetizer, stimulant, emetic, diuretic,and rubefacient	Leung, 1980
Brassica oleracea L., *B. rupa* L.	Plant	Rapine	Antifungal		Lewis and Elvin-lewis, 1977
Cheiranthus cheiri L.	Seeds	Cheirolin	Antibacterial, antifungal	Oral ulcers	

Table 1 (cont.)

Botanical origin	Part(s) used	Antimicrobial compound(s)	Biological activity	Ethnobotanic use	Reference
Raphanus sativus L.	Seeds and stems	Raphanin	Antibacterial (gram positive and gram negative bacteria)	-	Lewis and Elvin-lewis, 1977
Cannabidaceae					
Humulus lupulus L.	-	Lupulon, humulon	Antibacterial (gram positive and acid-fast bacteria)	Prevent bacteria from growing in beer or wort	
Compositae					
Artemisia sublessingiana Kell, Krasch et Poljak.	Aerial parts	Chryseriol, 5, 7, 4-trihydroxy-6, 3'-dimethoxy-flavone	Antifungal	-	Manadilova Adiyatova, and Kunaeva, 1987
Inula helenium L.	Dried roots and rhizomes	Helenin (alantolactone)	Antibacterial, anthelmintic	Treating asthma, bronchitis, whooping cough, nausea	Leung, 1980
Ericaceae					
Arbutus unedo L.	-	Ethyl gallate	Antibacterial (*Mycobacterium*)	-	Lewis and Elvin-Lewis, 1977

Table 1 (cont.)

Botanical origin	Part(s) used	Antimicrobial compound(s)	Biological activity	Ethnobotanic use	Reference
Flacourtiaceae					
Hydnocarpus anthelmintica Pier.	Seed oil	Hydnocarpic acid	Antibacterial (*Mycobacterium*)	Against leprosy	Lewis and Elvin-Lewis, 1977
Geraniaceae					
Pelargonium graveolens (L.) L'Her.ex Ait.	Fresh leaves and stems	Geranium oil	Antibacterial, antifungal	-	Leung, 1980
Gramineae					
Cymbopogon nardus (L.) Rendle, *C. winterianus* Jowitt	-	Citronella oil	Antibacterial, antifungal	Vermifuge, diuretic, stomachic, diaphoretic	Leung, 1980
C. citratus (DC.) Stapf	Fresh or partially dried leaves	Lemongrass oil	Antibacterial (gram positive bacteria), antifungal	Treat colds, headache, stomachache, abdominal pain	Leung, 1980
Guttiferae					
Garcinia kola Hekkel	Fruit pulp	Kolanone	Antibacterial, antifungal	-	Hussain et al., 1982
G. mangostana L.	Fruits	Mangostin	Antibacterial, antifungal	-	Sundaram et al., 1983
G. morella Desr.	Seeds	Morellin, guttiferins	Antibacterial, (gram positive and gram negative bacteria)	- -	Lewis and Elvin-lewis, 1977

Table 1 (cont.)

Botanical origin	Part(s) used	Antimicrobial compound(s)	Biological activity	Ethnobotanic use	Reference
Hypericaceae					
Hypericum drummondii (Grev. & Hook.) T. & G.	Roots, leaves and stems	Drummondins A, B, C, and F	Antibacterial (gram positive and acid-fast bacteria)	-	Jarasuriya et al., 1989
H. japonicum Thunb.	Whole plant	Sarothralen A, B	Antibacterial (against S. *aureus*, *Bacillus cereus* and *Nocardia gardenen*)	Treatment of several bacterial diseases, infections hepatitis, gastrointestinal disorders, and tumors	Ishiguro, Yamaki, Kashihara, and Takagi 1986
		Sarothralin G	Antibacterial (gram positive bacteria)		Ishiguro, Yamaki, Kashihara, Takagi, and Isoi, 1990
Juglandaceae					
Juglans regia L. and other species	Bark and fruit	Juglone	Antifungal	-	Lewis and Elvin-lewis, 1977

Table 1 (cont.)

Botanical origin	Part(s) used	Antimicrobial compound(s)	Biological activity	Ethnobotanic use	Reference
Labiatae					
Melissa officinalis L.	Leaves with flowering tops	Balm oil	Antibacterial (especially against *Mycobacterium phlei* and *Streptococcus hemolyticus*)	-	Leung, 1980
Mentha piperita L., *M. arvensis* L. var. *piperascens* Malinvaud	Dried leaves	Peppermint and cornmint oils	Antimicrobial, cytotoxic	Stomachic, antiseptic, local anesthetic and antispasmodic	Leung, 1980
Origanum vulgare L.	Dried herb and leaves	Thymol and carvacrol	Strong fungicidal, anthelmintic	Stimulant, carminative diaphoretic, and nerve tonic	Leung, 1980
Pogostemon cablin (Blanco) Benth.	Dried leaves	Dhelwangin (pogostone)	Antibacterial, antifungal	Treat colds, headaches, nausea, vomiting	Leung, 1980
Rosmarinus officinalis L.	Dried leaves (which supplies the spice)	Rosemary oil	Antibacterial, antifungal	Tonic, stimulant, carminative, stomach pains	Leung, 1980

Table 1 (cont.)

Botanical origin	Part(s) used	Antimicrobial compound(s)	Biological activity	Ethnobotanic use	Reference
Salvia officinalis L.	Dried leaves	Salvin and salvin monomethyl ether	Antibacterial	Tonic, digestive, antiseptic, astringent	Leung, 1980
Satureja hortensis L.	Dried leaves and tender stems	Summer salvory oil	Antibacterial, antifungal	Tonic, carminative, expectorant, astringent	Leung, 1980
Thymus capitatus (L.) Hoffmgg. et Link	Flowering tops	Carvacrol	Antifungal, anthelmintic	-	Leung, 1980
T. vulgaris L.	Dried leaves and flowering tops	Thymol and carvacrol	Antibacterial, antifungal, anthelmintic	Anthelmintic antispasmodic, carminative, expectorant, sedative	Leung, 1980
Lauraceae					
Cinnamomum zeylanicum Garc. ex Bl., *C. loureirii* Nees, and *C. burmanii* (Nees) Bl.	Dried bark, leaves, and twigs	Cinnamon oil	Antibacterial, antifungal, antiviral and larvicidal	Colds, rheumatism, and chronic diarrhea	Leung, 1980
Laurus nobilis L.	Dried leaves	Laurel leaf oil	Antibacterial, antifungal	Carminative and diaphoretic	Leung, 1980

Table 1 (cont.)

Botanical origin	Part(s) used	Antimicrobial compound(s)	Biological activity	Ethnobotanic use	Reference
Persea americana Mill.	Dried pulp of fruit, seeds	4,8"-Biscatechin	antitumor (against Sarcoma 180 in mice and Walker 256 in rats)	To hasten the suppuration of wounds, and for dysentery	Leung, 1980
Leguminosae					
Canavalia ensiformis DC.	Seeds	Canavanine	Antibacterial, antifungal	-	Lewis and Elvin-lewis, 1977
Dalbergia nigra Allem.	-	Dalbergions	Antibacterial (broad spectrum)	Used as anthelmintic and to remove pimples	
Haematoxylon campechianum L	Leaves	Ethyl gallate	Antibacterial (*Mycobacterium*)	-	
Medicago sativa L.	Aerial parts	Medicagol	Antifungal	A nutrient to increase vitality, appetite and diuretic	Leung, 1980
Melilotus spp.	Plant	Dicumarol	Antibacterial (broad spectrum)	used against colds	Lewis and Elvin-lewis, 1977

Table 1 (cont.)

Botanical origin	Part(s) used	Antimicrobial compound(s)	Biological activity	Ethnobotanic use	Reference
Ononis natrix L. subsp. *natrix*	Blossoms	Flavonoid	Antibacterial (gram positive bacteria)	-	Marhuenda, Raquena and Garcia Ginenza, 1986
Phaseolus vulgaris L.	Seeds	Phaseollin	Antifungal	-	Lewis and Elvin-lewis, 1977
Prosopis juliflora DC.	-	Juliflorine	Antibacterial (gram positive and gram negative bacteria)		Ahmad et al., 1986
Liliaceae					
Allium sativum L.	Bulb	Allicin, allistatin	Antibacterial (broad spectrum), antifungal	Treat tuberculosis, coughs, colds, and toochache	Lewis and Elvin-lewis, 1977
Aloe barbadensis Mill.	Plant	Barbaloin	Antibacterial (tubercle bacilli)	Against ringworm	
Lythraceae					
Lawsonia inermis L.	Dried leaves	Lawsone	Antibacterial, antifungal, antitumor	Treating skin problems, headache, amebiasis, and cancers	Leung, 1980

Table 1 (cont.)

Botanical origin	Part(s) used	Antimicrobial compound(s)	Biological activity	Ethnobotanic use	Reference
Magnoliaceae					
Magnolia grandiflora L.	Seeds	Magnolol, honokiol, 3,5'-diallyl-2'-hydroxy-4-methoxybiphenyl	Antibacterial (gram positive and acid-fast bacteria), antifungal	-	Clark, El-Feraly, and Li, 1981
Moraceae					
Brosimopsis oblongifolia Ducke	Roots	Cudraflavone B	Antibacterial (gram positive and acid-fast bacteria), cytotoxic against KB cells	-	Messana, Ferrari, and de Araujo, 1987
Maclura pomifera (RAF.) Schneider	Wood	Tetrahydroxystilbene	Antifungal (Cellulose decomposing fungi)	-	Lewis and Elvin-lewis, 1977
	Fruit	Osajin, pomiferin	Antibacterial (broad spectrum)	-	Mahmoud, 1981
Moringaceae					
Moringa oleifera Lam.	Roots	Pterigospermin	Antibacterial (gram positive and gram negative bacteria)	-	Lewis and Elvin-lewis, 1977
Myricaceae					
Myrica cerifera L.	Dried root bark	Myricitrin	Bactericidal, *Spermicidal*	-	Leung, 1980

Table 1 (cont.)

Botanical origin	Part(s) used	Antimicrobial compound(s)	Biological activity	Ethnobotanic use	Reference
Myrtaceae					
Eucalyptus globulus Labill.	Leaves	Eucalyptus oil and Eucalyptol	Antibacterial	Antiseptic, febrifuge, expectorant	Leung, 1980
Eugenia aromatica (L.) Baill.	Buds, stems, leaves	Eugenol	Antibacterial, antifungal	Carminative, antiemetic, and counte-rirritant	Leung, 1980
Piperaceae					
Piper cubeba L.f.	Dried, mature fruit	Cubeb oil	Antibacterial	Urinary anti-septic, carminative	Leung, 1980
Plumbaginaceae					
Plumbago europaea L. and other spp.	Roots	Plumbagin	Antibacterial, antifungal	Toochache, swellings	Lewis and Elvin-lewis, 1977
Polygonaceae					
Rheum officinale Baill.	Rhizome	Rhein (cassic acid)	Antibacterial (gram positive and acid-fast bacteria)	Vermifuge	
Pyrolaceae					
Chimaphila umbellata Nutt.	Dried leaves	Chimaphilin	Antibacterial, antifungal	Diuretic, astringent, diaphoretic, and mild disinfectant	Leung, 1980

Table 1 (cont.)

Botanical origin	Part(s) used	Antimicrobial compound(s)	Biological activity	Ethnobotanic use	Reference
Ranunculaceae					Lewis and Elvin-lewis, 1977
Anemone spp., *Ranunculus* spp.	Plant	Anemonin, protoanemonin	Antibacterial (broad spectrum)	Treat abrasions, toothache, rheumatism	
Hydrastis canadensis L.	Dried rhizome and root	Berberine	Antibacterial (broad spectrum) antiprotozoal	Antiperiodic, antiseptic, hemostatic	
Rosaceae					
Malus spp., *Prunus* spp., *Pylus* spp.	Bark and root	Phloretin	Antibacterial (gram positive and gram negative bacteria)	-	
Sorbus acuparia L.	Fruit	Parasorbic acid	Antibacterial (gram positive bacteria), antiprotozoal	Coughs and cattarrh	
Rubiaceae					
Cinchona succirubra Pav. ex Klotsch, *C. calisaya* Wedd. and their hybrids	Dried bark	Quinine	Antimalarial	Treating malaria, fevers, indigestion	Leung, 1980
Rutaceae					
Citrus bergamia Risso et Poit.	The peel of fresh, nearly ripe fruit	Bergapten, xanthotoxin	Antifungal	-	Leung, 1980

Table 1 (cont.)

Botanical origin	Part(s) used	Antimicrobial compound(s)	Biological activity	Ethnobotanic use	Reference
C. paradisi Macf.	Fresh peel of the fruit	Grapefruit oil	Antibacterial	-	Leung, 1980
Vepris louisii G. Gilbert	Trunk bark	Veprisinium salt	Antibacterial (gram positive bacteria)	Treating skin diseases of bacterial	Ayafor, sondengam, and Nga-djui, 1982
Salicaceae					
Populus candicans Ait.,	Bark	Trichocarpin	Antifungal	-	Lewis and Elvin-lewis, 1977
P. tacamahacca Mill.	Young shoots	Bisabolol	Antibacterial (Tubercle bacilli)	-	
Scrophulariaceae					
Capraria biflora L.	Root	Biflorin	Antifungal	-	
Solanaceae					
Lycopersicon pimpinelli-folium Mill.	-	Tomatine	Antibacterial (gram positive bacteria)	-	
Solanum tuberosum L.	Plant	Tuberosines	Antiprotozoal	Treat sores and for coughs	
Umbelliferae					
Anethum graveolens L.	Dried ripe fruit	Dill seed oil	Antibacterial	Aromatic carminative	Leung, 1980
A. sowa Roxb.		Indian dill seed oil	Antifungal		

Table 1 (cont.)

Botanical origin	Part(s) used	Antimicrobial compound(s)	Biological activity	Ethnobotanic use	Reference
Angelica archangelica L.	Root and rhizome	Angelica root oil	Antibacterial, antifungal	Bronchial ailments, colds, coughs and stomach trouble due to indigestion	Leung, 1980
Carum carvi L.	Dried ripe fruit	Caraway oil	Antibacterial, larvacidal	Antispasmodic, carminative, expectorant, stomachic	Leung, 1980
Centella asiatica (L.) Urban	Plant	Asiaticoside	Antibacterial (*Mycobacterium*)	Treat leprosy	Lewis and Elvin-lewis, 1977
Coriandrum sativum L.	Dried ripe fruit and leaves	Coriander oil	Larvicidal, bactericidal and weakly cytotoxic	Aromatic carminative, stomachic, antispasmodic	Leung, 1900
Cuminum cyminum L.	Dried ripe fruit	Cumin oil	Larvicidal, bactericidal	Stimulant, antispasmodic, carminative, diuretic	Leung, 1980
Foeniculum vulgare Mill.	Dried ripe fruit	Fennel oil	Antibacterial	Stomachic, carminative	Leung, 1980

Table 1 (cont.)

Botanical origin	Part(s) used	Antimicrobial compound(s)	Biological activity	Ethnobotanic use	Reference
Valerianaceae					
Valeriana officinalis L., *V. jatamansii* Jones	Dried rhizome and root	Valerian	Antibacterial (gram positive bacteria)	Antispasmodic, carminative, stomachic, sedative	Leung, 1980
Verbenaceae					
Lantana trifolia L.	Dried leaves	Umuhengerin	Antibacterial, antifungal	-	Rwangabo et al., 1988
Winteraceae					
Pseudowintera colorata (Raoul). Dandy	Leaves	Polygodial	Antifungal (*Candida albicans*)	Treat skin diseases, anagesic	Mc Callion et al.,
Zingiberaceae					
Alpinia galanga (L.) Willd.	Dried seeds	Galanal A,B, and galanolactone	Antifungal, cytotoxic KB cells	-	Morita and Itokawa, 1988
Curcuma longa L., *C. viridiflora* Roxb.	Root	Curcumin	Antibacterial (broad spectrum), antifungal	Used in India as anthelmintic	Lewis and Elvin-lewis, 1977

II. ***Eleutherine palmifolia* (L.) Merr.**

The plant belonged to genus *Eleutherine* Herbert., family Iridaceae. According to the Index Kewensis (Prain, 1911-1915; Hill, 1921-1925), Flora of Java (Backer and Bakhuizen Van Den Brink, 1968), and the Medicinal Plant of East and Southeast Asia (Perry and Metzager, 1980), the synonymous names of the species should be as followed:-

Eleutherine americana (Aubl.) Merr.
E. plicata Herb.
E. bulbosa (Mill.) Urb.
Sisyrinchium palmifolium L.
Antholyza meriana Blanco.

Local names of *E. palmifolia* (L.) Merr. were listed as followed :-

Bo-choe "บ่อเจอ", Phoh-bee-be "เพาะบีเบ" (Mae Hong Sorn)
Waan Kai daeng "ว่านไก่แดง", Waan Khao "ว่านเข้า",
Waan maak "ว่านหมาก" (Northern of Thailand)
Waan phloh "ว่านเพลาะ" (Chiang Mai)
Waan hom daeng"ว่านหอมแดง" (Central of Thailand)
(Tem Smitinand, 1980)

E. palmifolia (L.) Merr. was a native plant of tropical America (Quisumbing, 1951). it was cultivated and naturalized in several localities in Indonesia, Philippines, Hainan Island of south China and some parts of Thailand.

The floral sheaths 2-10 together in the axils of the 1-3 highest cauline leaves; lowermost cauline leaf well-developed, erect, higher ones bract-like; peduncle erect or more or less patent, often curved or sinuous, 2 $^{1}/_{4}$-4 cm; floral sheaths 12-16 mm long, green, 4-10 flowered; pedicles 1-1 $^{1}/_{2}$ cm,

enclosed within the sheaths; flowers ephemerous, inodorous; perianth bright white, 1 $^{1}/_{2}$-3 $^{1}/_{2}$ cm across; tepals oblong, obvate or spatulate, inner ones smaller ; anthers and filaments bright yellow; style-arms erecto-patent, alternating with the anthers, yellow. Leaves glabrous, 25-60 cm by 1-2 $^{1}/_{2}$ cm. Stem erect, obliquely erect, drooping or documbent, often sinuous; bulb elongately ovoid, red 0.25-0.50 (Backer and Bakhuizen Van Den Brink, 1968).

E. palmifolia (L.) Merr. was used as a folk medicine in many countries, mostly in Asia. In Indonesia, the tuber was considered to be diuretic, purgative, and emetic; it was prescribed to treat dysentery, inflammation and prolapse of rectum.

In Philippines, the crushed bulb was applied to the stings of poisonous fish, to charley horse and to draw a thorn from the foot, also to insect stings, wounds, and boils. The roasted tuber was on the abdomen to treat pain. (Perry and Metzger, 1980)

In China, the red bulb was used to treat cardiac disease, especially coronary disorders. (Hainan-Renmin Hospital Guanxinbin-Keyan-Xiaozu, 1977 quoted in Chen, Huang, Wang, Li, Ding, et al., 1984)

In Thailand, the bulb was used for carminative and decongestant in children. (Sophit Dhamaree and Monthira Tungayoon, 1982)

In Haiti, this plant was widely used as antifertility agent (Weniger, Haag-Berrurier, and Anton, 1982).

Figure 1 *Eleutherine palmifolia* (L.) Merr.

(ว่านหอมแดง) , Iridaceae

A. Chemical constituents

There were four naphthalene derivatives and three anthraquinones which isolated from *E. palmifolia* (L.) Merr. bulb.

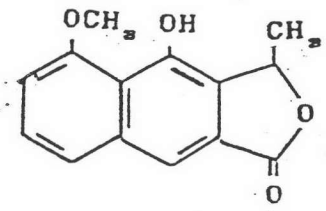
In 1950 (Schmid, Meijer, and Ebnöther; Schmid, Ebnöther, and Burger), the first naphthalene derivative from *E. palmifolia* (L.) Merr. bulb was reported. It was eleutherol.

In 1951 Schmid and Ebnöther reported another two naphthalene derivatives, they were eleutherin and isoeleutherin.

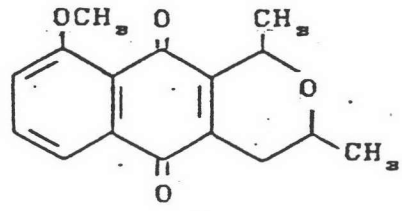
Komura and others (1983) isolated three new minor anthraquinone pigments from ethanol extract of *E. palmifolia* (L.) Merr. and their structures were elucidated as methyl ethers of 3,4,8-trihydroxyl-1-methyl-anthra-9,10-quinone-2-carboxylic acid methyl ester by spectral analysis including long-range selective proton decoupling (LSPD) experiments in ^{13}C-NMR.

In 1984, Chen, Huang, Wang, Li, Ding, and others isolated hongconin, a new naphthalene derivative, from *E. palmifolia* (L.) Merr. bulb. The structure of hongconin was determined by spectral and x-ray analyses.

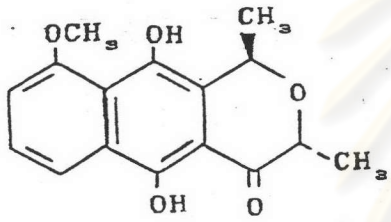
Figure 11 Chemical constituents isolated from *Eleutherine palmifolia* (L.) Merr. bulb



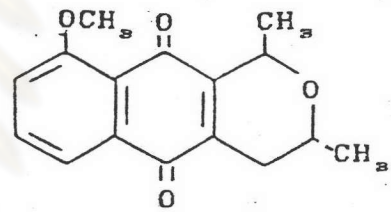
Eleutherol



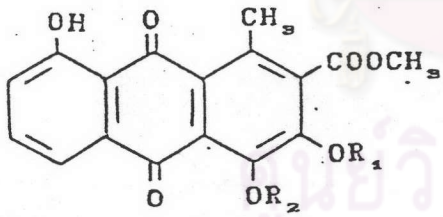
Eleutherin



Hongconin



Isoeleutherin



Methyl ethers of 3,4,8-trihydroxyl-1-methyl-anthra-9, 10-quinone-2-carboxylic methyl ester

R_1	R_2
$-CH_3$	$-H$
$-H$	$-CH_3$
$-CH_3$	$-CH_3$

B. Pharmacological investigations

The pharmacological reports of *E. palmifolia* (L.) Merr. were concentrated on naphthalene derivatives extracted from bulb.

In 1975, Bianchi and Ceriotti reported general pharmacological properties of eleutherol and eleutherin *in vivo*; eleutherol was given intraperitoneally to six groups of mice (5 animals/group) at various doses (800, 400, 200, 100, 50 and 25 mg/kg) without provoking overt toxic effects within 3 hours of observation. It only provoked abdominal writhing at all doses and hypothermia at 800 and 400 mg/kg. The central nervous system, the automatic nervous system, and the muscular system were not affected by this compound as judged by a direct observation of the treated mice.

Eleutherin was given intraperitoneally to groups of mice (5 animals/group) at 400 mg/kg, it provoked the death of one animal; reduced spontaneous motility and curiosity; did not provoke straub tail, mydriasis, salivation, lacrimation, diuresis, diarrhea, and pilaerection; and did provoke abdominal writhings, twitches, and convulsions. At the same dose, it provoked hypothermia, motor incoordination, and analgesia. At 200 mg/kg and at lower doses down to 25 mg/kg, it produced abdominal writhings in all mice, with the effect lasting about 30 min.

Eleutherin, given intraperitoneally at 200 mg/kg to groups of mice, did not show anticonvulsant properties (after pentylenetetrazol (125 mg/kg sc), strychnine (3 mg/kg sc), and electric seizures (15 mamp for 0.2 sec)), antichemotremor properties (oxotremorine tremors), and antireserpine properties (reserpine ptosis and hypothermia). It did not

modify the hypnotic effects of pentobarbital, the stimulant properties of amphetamine in aggregated mice (toxicity and hypermotility), the parasympathetic stimulant properties of oxotremorine and carbacholine, the lethal effects of epinephrine, and the intestinal motility (Bianchi and Ceriotti, 1975).

When given 100 mg/kg per oral to groups of rats (10 animals/group), did not show anti-inflammatory or analgesic effects in edematous hyperalgesia. Elutherin, 200 mg/kg po and ip, shorten the Quick prothrombin time from the control value of 13.2 to 8.3 sec (po) and to 8.1 sec (ip). The effect was manifested 15 min after treatment but not 30 min and 1, 4 and 24 hr after treatment. 100 mg/kg ip, increased blood pressure 15 and 30 min after dosing (initial blood pressure of 175 mmHg; increase of 15-20 mmHg); blood pressure returned to normal within 60 min.

Eleutherin, 300-600 µg/kg iv given to three male rabbits, provoked an occasional and modest fall in blood pressure which lasted in only a few second only, but it failed to modify blood pressure considerably and for long periods. It did not alter the responses of the animals to epinephrine, acetylcholine, and carotid occlusion. At higher doses (2 mg/kg), it provoked the arrest of respiration and death (Bianchi and Ceriotti, 1975).

In 1981, Chen, Huang, Wang, Li, and Ding reported three aromatic compounds, eleutherol, eleutherin and isoeleutherin as coronary vasodilating in isolated guinea pig heart. Ding and Huang (1982) prepared the tablet which contained these three active constituents of *Eleutherine palmifolia* (L.) Merr. bulb for treatment of heart diseases

such as angina pectoris.

Weniger and others (1982) reported that *E. palmifolia* (L.) Merr. which used as antifertility agents in Haiti, showed the activity to the uterus but was toxic.

C. Antibacterial and Cytotoxic activity

There was only one report about the antibacterial and cytotoxic activity of chemical compounds from *E. palmifolia* (L.) Merr. Bianchi and Ceriotti (1975) tested eleutherol and eleutherin at the concentrations ranging from 7.17 to 125 µg/ml. They found that eleutherol failed to inhibit the development of *Bacillus subtilis* (ATCC 9466) up to 125 µg/ml with the agar plate diffusion method, while eleutherin was found to inhibit the development at various concentration down to 62.5 µg/ml. Both of these compounds, up to 125 µg/ml, did not show any antidehydrogenase activity on Sarcoma 180 cells.

III. **Activities of naturally occurring naphthoquinones**

The distribution of the naphthoquinone was sporadic. Nearly half of them occurred in higher plants, scattered through some twenty families. They have been found in leaves, flowers, wood, bark, roots and fruits. The naphthoquinones were also found in fungi and bacteria but for animals only echinoderms were known to elaborate naphthoquinones (Thomson, 1971).

During a search for new antibiotics, several plants and fungi were found to produce antimicrobial substances which led to subsequent chemical and activity investigations. Some of these compounds showed the chemical structure of 1,

4-naphthoquinone. There were many studies reported on the activity of naphthoquinone *in vitro* and *in vivo*.

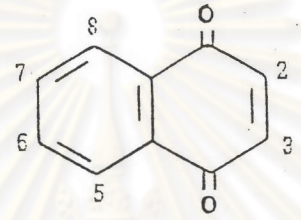
A. Antibacterial and antifungal activity

In 1964, Oka reported that the antimicrobial effect of 1,4-naphthoquinone and 2-methyl-1,4-napthoquinone on yeast cells, *Escherichia coli*, and *Staphylococcus aureus* which he said it depended upon cell permeability.

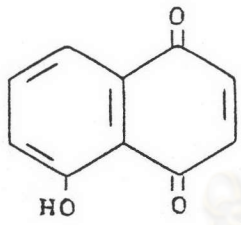
In 1966, Rein, Vlad and Aizenberg studied on antimicrobial properties of juglone (5-hydroxy-1,4-naphthoquinone) and its derivatives; 2-(ethanolamino) juglone, 3-(diethanolamino)juglone, 3,6-dibromo-2-(diethanolamino)juglone and 2,6-dibromo-3-(diethanolamino) juglone against gram positive and gram negative bacteria using broth dilution method and disk susceptibility test. They found that juglone was active against *Bacillus anthracoides*, *B. cereus*, *B. anthracis*, and *B. brevis* at dilution of 1:200 while 2-(ethanolamino) juglone, 3-(diethalamino) juglone, 3-6-dibromo-2-(diethanolamino) juglone, and 2,6-dibromo-3-(diethanolamino)juglone were active against *B. cereus* var *mycides*, *B. anthracoides*, *B. cereus*, and *B. mecentericus* at the dilutions of 1:6400-1:1600, 1:3200-1:800, 1:1600-1:400, and 1:1600-1:400, respectively.

One year later, Ikekawa and others (1967) extracted juglone from walnut pericarp. The determination of the minimal inhibitory concentrations for a wide variety of bacteria, yeasts and fungi were performed. The MIC_s of the extract against various microorgnisms were as followed; *Micrococcus flavus* 3 µg/ml; *Corynebacterium xerosis*, *Candida pseudotropicalis* 12 µg/ml; 25µg/ml inhibited the growth of *Bacillus subtilis*, *Trichophyton mentagrophytes*,

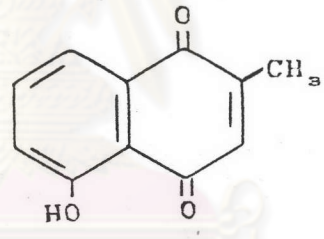
Pellicularia filamentosa (Rhizoctonia filamentosa); 50 µg/ml inhibited the growth of *Salmonella paratyphi* A, *Staphylococcus aureus*, *Piricularia oryzae*, *Selerotium rolfsii*, *Ophiobolus miyabeanus*, and *Helminthosporium sesanum*; and $\geq$ 100 µg/ml inhibited a variety of other intestinal, soil, or respiratory tract microorganisms, yeasts and fungi.



1,4-naphthoquinone



Juglone



plumbagin

Oswaldo and others (1968) purified plumbagin(2-methyl juglone) from alcohol extract of *Plumbago scandens* L. dry leaves using column chromatography and observed antimicrobial action of plumbagin against bacteria and fungi. Plumbagin inhibited the growth of *Bacillus subtilis* at the concentration of 5 µg/ml; 5-10 µg/ml inhibited the growth of *Brucella suis*, *B. abortus*, and *B. melitensis*; 50-100 µg /ml inhibited the growth of *Candida albicans*; and *Penicillium lilacinum* was inhibited at the concentrations between 10-20 µg/ml. Plumbagin was also extracted from the roots of *Plumbago auriculata* Lamk and *Plumbago zeylanica* L. (Van der Vijver and Loetter, 1971). The

antibacterial activities were tested by applying plant fragments to the cultures of *Klebsiella aerogenes* (*Enterobacter aerogenes*), *Staphyloccus aureus*, *Bacillus pumilus*, and *B. cereus* which showed strong activity (Van der Vijver and Loetter, 1971).

As in the report of Krishnaswamy and Purushothaman (1980), plumbagin inhibited the growth of both gram positive and gram negative bacteria by adding substance to the nutrient agar medium and observed growth of the organism for 48 hr. Antifungal activity was studied by adding the similar doses of plumbagin in sabourauds medium. The results were shown in Table 2.

Table 2 Antibacterial and antifungal activities of plumbagin

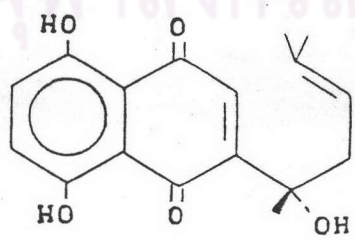
	0	10 ug/ml	20 ug/ml
Staphylococcus aureus	+++	+++	-
Staphylococcus albus	+++	++	-
Salmonella paratyphi	+++	++	-
Coryebacterium equi	+++	+	-
Klebsiella pneumoniae	+++	+	-
Staphylococcus citreus	+++	++	-
Salmonella dublin	+++	++	-
Rhizopus nigricans	+++	-	-
Epidermophyton floccosum	+++	-	-
Microsporum nana	+++	-	-
Penicillium notatum	+++	-	-
Penicillium canadense	+++	-	-

Complete inhibition of growth was observed from 20 ug/ml onwards

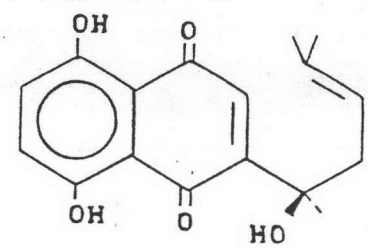
+, Growth ; -, No growth

(Krishnaswamy and Purushothaman, 1980)

The root of *Alkanna tinctoria* Tausch (Boraginaceae) was used for dyeing since antiquity. The principal pigments of the roots were naphthoquinones. When a benzene or hexane extract of *A. tinctoria* Tausch was examined, no free alkannin could be identified. Instead of free alkannin, its β,β-dimethylacrylic ester and β-acetoxy isovaleric ester of alkannin were identified to be the principal pigments of this plant (Papageogiou, 1977). Because shikonin had the (R)- and alkannin the (S)-configuration, shikonin had been tested for its antibacterial acitivity (Tanaka and Odani, 1972 quoted in Papageogiou, et al., 1979). This observation prompted Papageogiou and others (1979) to provide an assessment of the antimicrobial properties of various constituents of the root of *A. tinctoria* Tausch and they found that alkannin and its esters, β,β-dimethylacrylic ester and β-acetoxyisovaleric ester, produced complete inhibition of *Staphylococcus aureus* and S. *epidermidis* when tested by the disc method. But these compounds exhibited no antibacterial properties against *Escherichia coli*. Of the three, only alkannin inhibited the fungus, *C. albicans*.

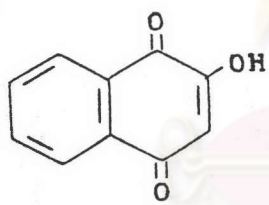


alkannin

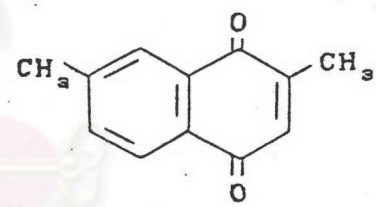


shikonin

Comparative the inhibitory effects among plant naphthoquinone was done in 1972 on three yeast species, three species of lactic acid bacteria, and four species of acetic acid bacteria (Shcherbanovskii et al., 1972). Juglone, isolated from fruits of *Juglans regia* L. and *Carya pecan* Engler and Graebn. showed the strongest inhibitory effect while its isomers lawsone, isolated from *Lawsonia inermis* L. leaves, and chimaphilin, isolated from *Pyrola secunda* L. leaves, showed the weakest effects. The inhibitory effect of shikonin, isolated from *Echium rubrum* Forsk. roots was moderate. They concluded that fungicidal and bactericidal activities of juglone and shikonin were greater than sulfur dioxide.



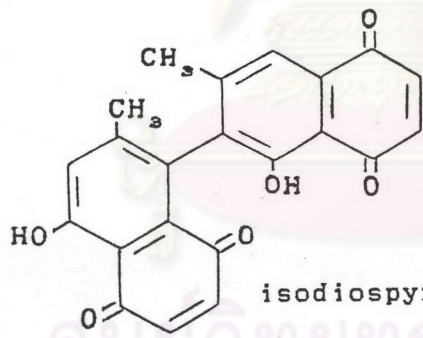
lawsone



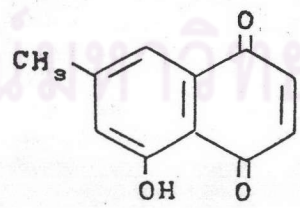
chimaphilin

The antimicrobial activity of plumbagin, juglone and lawsone were determined again in 1986 (Didry, Pinkas, and Dubreuil). They presented that *C. albicans* was sensitive to juglone and plumbagin while staphylococci, enterococci, and *Acinetobacter calcoaceticus* were only sensitive to plumbagin. Some activity against anaerobes was observed too; *Bacteroides fragilis* was sensitive to juglone and *Propionibacterium acnes* to juglone and plumbagin.

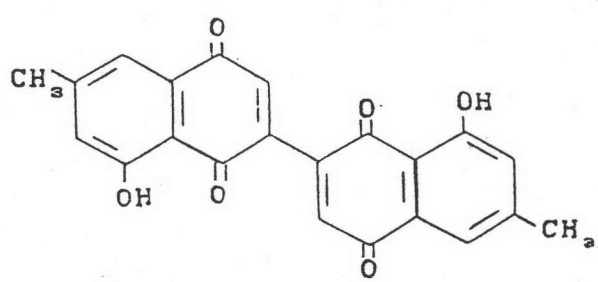
Extraction of *Diospyros usambarensis* A. DC. (Ebenaceae) root bark with petroleum ether by flash chromatography yielded two naphthoquinones. These were characterised as 7-methyljuglone and isodiospyrin. A further dimeric naphthoquinone, mamegakinone was isolated from the chloroform extract and with methanol extract, it gave 2-methoxy-7-methyl juglone and 3-methoxy-7-methyl juglone (Marston, Msonthi, and Hostettmann, 1984). The fungicidal activity was ascertained by a TLC assay, using spores of *Cladosporium cucumerinum*. In this assay 0.025 µg of 7-methyl juglone was sufficient to prevent growth of the fungus. Isodiospyrin, 2-methoxy-7-methyljuglone and 3-methoxy-7-methyl juglone in 10 µg amounts also proved to be fungicide but 10 µg of mamegakinone was inactive.



isodiospyrin

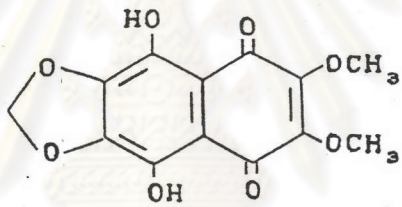


7-methyl juglone



mamegakinone

Tricrozarin A (5,8-dihydroxy-2,3-dimethoxy-6,7-methylenedioxy-1,4-naphthoquinone) was extracted with methanol from the fresh bulbs of *Tritonia crocosmaeflora* Lemoine (Iridaceae). It showed antimicrobial activity against *Bacillus subtilis* PCI 219 (MIC 5.1 µg/ml), *Micrococcus luteus* ATCC 9341 (MIC 112.0 µg/ml), *Aspergillus niger* ATCC 6275 (MIC 81.3 µg/ml), *Mucor racemosus* IFO 5403 (MIC 10.2 µg/ml), *Candida albicans* KF1 (MIC 81.3 µg/ml), and *Saccharomyces sake* KF 26 (MIC 81.3 µg/ml). (Masuda et al., 1987)



tricrozorin A

In 1988, Thatree Phadungcharoen and others isolated 2-methoxy-1,4-napthoquinone from the chloroform extract of Garden Balsam leaves (*Impatiens balsamina* L.). MIC and MFC of this compound against *Trichophyton rubrum*, *T. mentagrophytes* and *Microsporum gypseum* were 2.50 µg/ml whereas *Epidermophyton floccusum* and *Candida albicans* were 1.25 µg/ml.

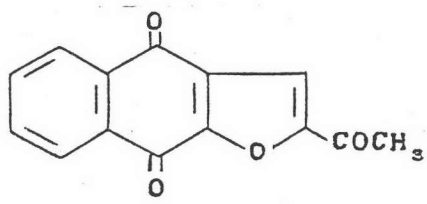
In vivo, the quinone plumbagin from *Plumbago europaea* L. was tested for tuberculostatic activity in mice (Vichkanova, Makarova, and Gordeikina, 1972). This compound was inactive against *Mycobacterium tuberculosis* and was

irritating in chemotherapeutic doses when tested *in vivo* against gram positive and gram negative bacteria, influenza virus, pathogenic fungi, and parasitic protozoa (Vichkanova, Adgina et al., 1972). The authors suggested the use of plumbagin in tropical therapeutic forms with mitigrated irritant action. For Microsporum infections in guinea pigs could be treated by local applications of 0.25-0.5% solutions (in 40% alcohol) or 1% emulsions of plumbagin.

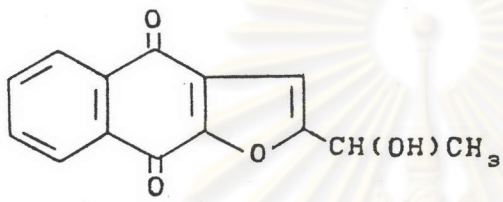
B. Anticancer and cytotoxic properties

In 1980, Krishnaswamy and Purushothaman studied for anticancer activity of plumbagin. Fibrosarcoma in rats (induced by methyl cholanthrene) was used for primary screening of potential anticancer drug. It was also tested against P_{388} lymphocytic leukaemia and L_{1210} lymphoid leukaemia. 70% and 60% regression of tumor (fibrosarcoma) were brought about when 2 mg/kg body weight plumbagin was given intratumour and orally. The ED_{50} of plumbagin for fibrosarcoma in rats was found to be 0.75 mg/kg body wt. For P_{388} lymphocytic leukaemia, plumbagin was active at 4 mg/kg body wt. It showed no activity against L_{1210} lymphoid leukaemia.

In the same year, *Tabebuia cassinoides* (LAM.) DC. (Bignoniaceae) stem bark was isolated to yield two new cytotoxic naphthoquinones. The structures were determined by spectroscopic means (UV, IR, ^{1}H-NMR and mass spectroscopy) to have the acetyl furonaphthoquinone and dihydro derivatives, $C_{14}H_8O_4$ and $C_{14}H_{10}O_4$. Both of them showed activity in the KB cell culture assay with ED_{50} 1.0 and 2.0 µg/ml respectively (Kingston and Adgina, 1980).

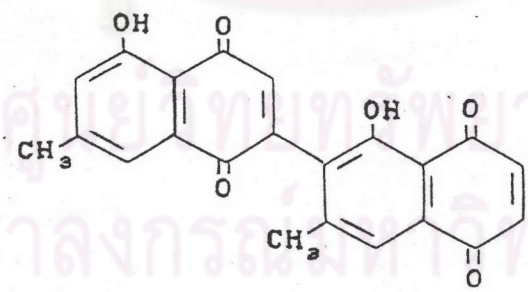


acetyl furonaphthoquinone structure



dihydro derivative of acetyl furonaphthoquinone

Hazra and others (1984) studies biological activity of diospyrin which was isolated from the stem bark of *Diospyros montana* Roxb. (Ebenaceae) against Ehrlich Ascites Carcinoma (E.A.C.) in Swiss Albino mice. The experiments were done both *in vitro* and *in vivo*.



diospyrin

In vitro, the cell-suspensions, treated with diospyrin, were examined under a phase-contrast microscope after 3 hr of incubation. Diospyrin was found to be highly cytotoxic towards E.A.C. cells at very low doses as compared to the control cells, as shown in Table 3.

Table 3 *In vitro* cytotoxicity of diospyrin towards E.A.C. cells (1 x 10^6 cells/ml)

Concentration of diospyrin in suspension (µg/ml)	Viability of E.A.C. cells (% of control) at		
	1 hour	2 hours	3 hours
5	88	48	7
10	67	26	0
20	10	0	0
20	10	0	0
40	0	0	0
80	0	(Total lysis occurred)	

(Hazra et al., 1984)

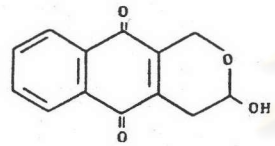
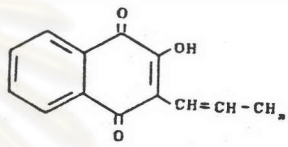
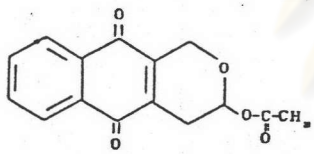
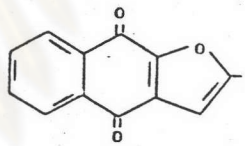
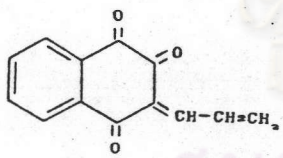
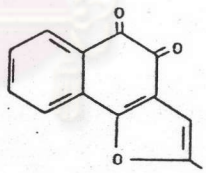
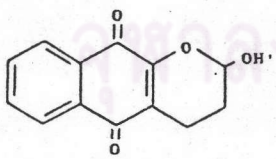
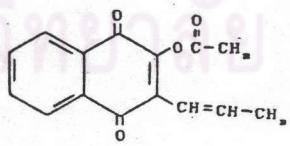
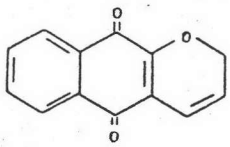
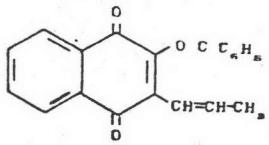
It was also found that after 90 min of incubation in presence of 0.2 mg of diospyrin the extent of inhibition of endogenous respiration of 6 x 10^7 E.A.C. cells was 72% with respect to the control (Hazra et al., 1984).

In vivo, the experiments showed the significant growth inhibition of E.A.C. and an increase in the life-span of the tumour bearing mice. This confirmed the effect of diospyrin against E.A.C. The pathologic significance of diospyrin in the treatment of E.A.C. bearing Swiss mice was obtained in the haematological studies. (Hazra et al., 1984)

Psychorubrin, a new naphthoquinone isolated from alcoholic extract of *Psychotria rubra* Poir. ground stem, showed sigificant reproducible inhibitory activity against KB cells (Hayashi, Smith, and Lee, 1987). The cytotoxicity of

Psychorubrin and related naphthoquinone derivatives in KB cell culture assay were performed as shown in Table 4.

Table 4 Cytotoxicity in the KB cell culture assay of Psychorubrin and related naphthoquinone derivatives

Compound	ED_{50} (µg/ml)	Compound	ED_{50} (µg/ml)
	3.0 Psychorubrin		14.0
	5.0		0.3
	0.4		0.6
	20.0		5.1
	0.4		5.1

(Hayashi, Smith, and lee, 1987)

Hayashi and others (1987), suggested that the conjugation originally presented in 1,4-naphthoquinone had been extended in all of the most active compounds, however it was not sufficient for good *in vitro* activity. They also observed that when a hydrophilic hydroxy group was presented in such compounds, the *in vitro* activity was reduced.

Plumbagin was found to increase the phagocytosis of human granulocyte *in vitro* in a concentration range of 2.5 pg - 25 pg/ml, whilts hydroplumbaginglucoside enhanced the proliferation of T-lymphocytes *in vitro* in a concentration range of 100 pg - 1 pg/ml. These two compounds were isolated, from *Dionaea muscipula* E. (Droseraceae), and tested by Kreher and others (1989).

C. Other activities

1. Antiprotozoal activity

Diospyrin exhibited antiprotozoal activity towards *Leishmania donovani*, the causative agent of kala-azar which existed in the phlebotomine sandfly as a flagellate extracellular promastigote and in human as a non-flagellate amastigote in macrophges, in culture. The minimal inhibitory concentration of diospyrin was 1 µg/ml in liquid culture medium, while almost total inhibition of respiration was observed with 5 µg/ml of diospyrin. With microscopic observations, the treated cells were loss of morphological characteristics which confirmed that diospyrin had a leishmanicidal activity (Hazra, Saha et al., 1987).

2. Molluscicidal activity

Preliminary screening of plant extracts for molluscicidal activity was reported in 1980 (Adewunmi and Sofowora). In many of the tested plant extracts, the

activity was probably due to the presence of a chalcone (Maradufa and Ouma, 1978), a flavonoid glycoside (Dossaji and Kubo, 1980), and saponins (Hostettmann, Kizu, and Tomimori, 1982). However, not only these compounds posesed molluscicidal properties, but also some naphthoquinones. The minimum concentration of 7-methyljuglone in water lethal to *Biomphalaria glabrata* snails, one of the vectors of schistosomiasis, within 24 hr was 5 ppm which represented a very efficient naturally occurring molluscicide. Plumbagin was active at 2 ppm, juglone at 10 ppm, isojuglone at 50 ppm, vitamin K3 at 3 ppm and lapachol was inactive at 50 ppm (Marston et al., 1984). The authors suggested that introduction of a hydroxy substituent into the quinoid ring (isojuglone, lapachol) instead of the aromatic ring caused a significant decrease in activity.

3. Antigermination activity

In 1986, Spenser and others studied on the effect of naturally occurring naphthoquinones on velvetleaf (*Abutilon theophrasti* Medik.) germination. They concluded that the more highly substituted compounds had little or no activity, and larger side chains tended to decrease effectiveness. Structures of the compounds tested and their antigermination activities against velvetleaf were shown in Table 5.

Table 5 Structure and activity of naphthoquinones

1,4-Naphthoquinones	Substitution	Concentration mM(%germination)*			
a 1,4-Naphthoquinone	none	0.6(96)	0.9(69)	1.2(28)	2.5(3)
b Menadione	2-CH_3	0.7(92)	0.9(61)	1(47)	1.2(3)
C Juglone	5-OH	0.4(100)	0.8(74)	1(41)	3(6)
d Lawsone	2-OH	1.2(97)	1.8(71)	2.1(33)	2.5(10)
e 2-Methoxy	2-OCH_3	0.2(96)	0.4(70)	0.5(52)	0.6(10)
f 5-Methoxy	5-OCH_3	2(100)	3(84)	471)	6(5)
g 2-Acetoxy	2-$OCOCH_3$		3(92)	4(51)	5(26)
h Plumbagin	2-CH_3-5-OH	0.2(100)	0.5(66)	0.7(22)	0.8(12)
i 2-Methyl-5-methoxy	2-CH_3-5-OCH_3	1(98)	2(58)	4(27)	5(0)
j 7-Methyljuglone	5-OH 7-CH_3		4(100)	6(45)	8(5)
k Phthiocol	2-OH 3-CH_3	1(93)	2(68)	4(18)	6(0)
l 2-Methoxy-3-methyl	1-OCH_3 3-CH_3	0.8(92)	1(68)	2(21)	4(0)
m Chimaphilin	2-CH_3 7-CH_3		1(90)	2(55)	4(8)
n 2,3-Dimethyl	2-CH_3 3-CH_3				10(100)
o 6,7-Dimethoxy	6-)CH_3 7-OCH_3				10(100)
p Lapachol	2-$CH_2CH=C(CH_3)_2$ 3-OH			10(100)	
q Isolapachol	2-$CH=CHCH(CH_3)_2$ 3-OH			10(100)	
r Lomatiol	2-$CH_2CH=CCH_3CH_2OH$ 3-OH		4(97)	6(77)	8(55)
s Vitamin K_1	2-$CH_2CH-CCH_3(CH_2CH_2$ $CH_2CHCH_3)_3CH_3$ 3-CH_3			10(100)	
t Naphthazarin	5-OH 8-OH			10(100)	
u Shikonin	2-$CHOHCH_2CH=C(CH_3)_2$ 5-OH 8-OH			10(100)	
v Cordeauxiaquinone	2-OH 3-CH_3 5-OH 6-CH_3 7-$COCH_3$ 8-OH			10(100)	
w Isodiospyrine	5-OH 7-CH_3 8-j			10(100)	
x Cassumunaquinone	2-$OCH_3$8-2,3-dimerthoxy phenyl			10(100)	

* Percent germination as compared to control. Figures below 85 are significantly different from controls at the 95% level by the Chi-square 1-tailed test.

(Spenser et al., 1986)