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Use of Secondary Metabolite in Tuberculosis: A Review

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ABSTRACT

Over few years research has been done for the effective treatment against the mycobacterium tuberculosis .Now a day's emerging multiple drug resistance has become a major threat and this lead to urgent requirement for new and effective treatments for this deadly disease .This review covers most recent report of natural occurring compound from plants and marine organism that show anti mycobacterial activity. Also consists of traditional medicinal uses of specific plants when utilized to treat tuberculosis and other pulmonary diseases. 17,500 plant species occurring in India, of which only about 365 species have been verified so far for antimycobacterial activity and the 255 (70% of 365) plant species from a wide range of families that belongs to different metabolites have shown antimycobacterial activity. Species are describes in tabular form family and plant part used, type of extract and in vitro activity (MIC value).

Key words: Tuberculosis, Antituberculer drugs, Mycobacterium tuberculosis, Secondary metabolites.

INTRODUCTION

Tuberculosis (TB) is currently the leading infectious diseases killing worldwide and it is assumed that *Mycobacterium tuberculosis* (bacteria), the causative agent of tuberculosis has infected one-third of the world's population [1]. Current antituberculosis treatments process a long course of a combination of antibiotics and toxic side effects and lead to poor patient compliance. There is now a need to discover and develop new safe and herbal antituberculosis drugs particularly to target drug resistance and improve the treatment of chronic tuberculosis by targeting tubercle bacilli which are thought to remain within the lungs in a non-replicating state of persistence and grows after some interval of time [2]. The organisms responsible for the disease are the bacteria (tubercle bacilli) - *Mycobacterium tuberculosis*, Mycobacterium tuberculosis complex including *mycobacterium bovis* and *mycobacterium africanum* [3].

The genus Mycobacterium (Mycobacteriaceae) is highly diverse with 85 species known [4]. Tuberculosis is largely a disease of poverty with the highest cases of the disease occurring in

Africa and Asia [5]. In sub-Saharan Africa 9 countries recently reported estimated annual incidences over 600 cases per 1, 00, 000 peoples. [6]. Naturally occurring pure compounds as well as extracts from different species of plants, microorganisms and marine organisms have indicated that inhibitory activity against Mycobacterium tuberculosis is widespread in nature so they can be used in tuberculosis treatment[7,8]. Natural products isolated from different plants have played an important role in discovery of drugs against infectious diseases like tuberculosis. Almost 75% of the approved anti-infective drugs are derived from medicinal plants and rest are synthesis chemically [9]. Over 350 plant species used in traditional medicine have been assessed for their antituberculosis activities [10]. The persistent increase of tuberculosis in Asia and Africa region may largely be attributed to the AIDS (Acquired Immune Deficiency Syndrome) pandemic combined with lower healthcare systems [11] .This estimated 1.7 million people who died of tuberculosis in 2006 [12].

Pathophysiology of tuberculosis (Fig.1)

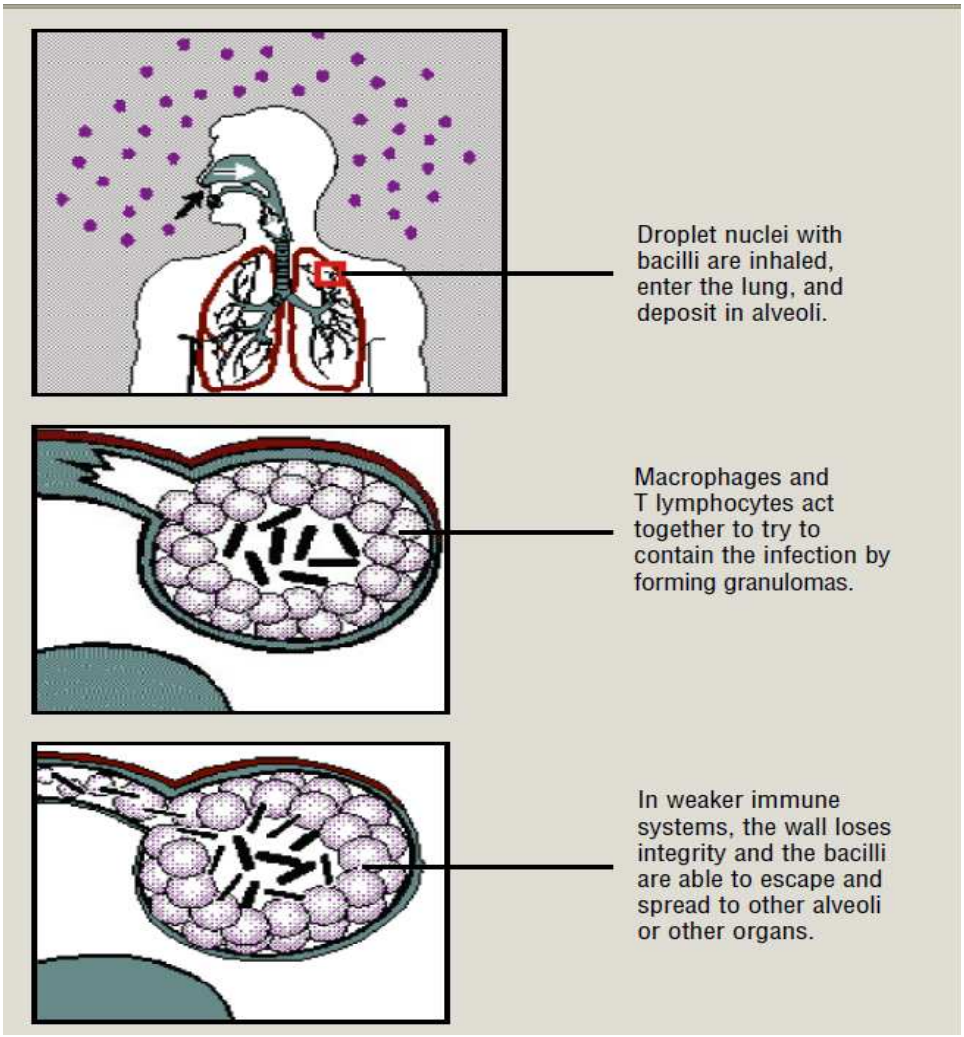


Fig: 1- pathophysiology of tuberculosis [15]

India, Asia and Africa represented by rich culture, traditions and natural biodiversity offer unique opportunity for the drug discovery researchers. Many species are used to isolate the antimycobacterial activity. This lead the development of safe and effective treatment of tuberculosis through herbal drugs [13]. This emphasizes that natural occurring compound isolated from the different plants with the structural diversity containing antituberculosis

properties with the minimal inhibitory concentration which is less than 200 µg/ml. This include the different classes of compound, such as saponins, steroids peptides, alkaloids, coumarins and flavones, alcohols obtained from plants, marine organisms, fungi and one bacterium[14].

As the cellular processes occur, tuberculosis may develop differently in each patient, according to the status of the patient's immune system. Stages include latency, primary disease, primary progressive disease and extrapulmonary disease. Each stage has different clinical manifestations (Table-1) [15].

Table No.1:-Different stages of tuberculosis [15]

Early infection	**Early primary progressive (active)**	**Late primary progressive (active)**	**Latent**
Immune system fights infection	Immune system does not control initial infection	Cough becomes Productive	Mycobacteria persist in the Body
Infection generally proceeds without signs or symptoms	Inflammation of tissues ensues	More signs and symptoms as disease progresses	No signs or symptoms occur
Patients may have fever, Paratracheal lymphadenopathy, or dyspnea	Patients often have nonspecific signs or symptoms (eg, fatigue, weight loss, fever)	Patients experience progressive weight loss, rales, anaemia	Patients do not feel sick
Infection may be only subclinical and may not advance to active disease	Non-productive cough develops	Findings on chest radio - graph are normal	Patients are susceptible to reactivation of disease
	Diagnosis can be difficult: findings on chest radiographs may be normal and sputum smears may be negative for mycobacteria	Diagnosis is via cultures of Sputum	Granulomatous lesions calcify and become fibrotic, become apparent on chest radiographs Infection can reappear when immunosuppression occurs

Tuberculosis is a wind-borne disease. It is transmitted from an infected person to another in the following manner:-

Coughing, Sneezing, Spitting, Discharging mucus, Kissing [16]

Herbal Aids Helping in Treatment for Tuberculosis

Some of the herbal plants used in the treatment of tuberculosis as homemade therapy. They are describing according to their dose use daily and there active contents present which use as anti tuberculosis agents.

Table No. 2: Antituberculosis therapy with natural substances [17, 18]

Name	Dose	Uses
Barberry *(Berberis vulgaris*)	one can include 10 to 15 barberry berries in the patients' diet daily	Barberries contain berberine, which has bactericidal properties and aid in killing the tuberculosis-causing bacteria.
Orange Juice	orange juice with a pinch of salt, a spoonful of honey, and two-three mint leaves	Vitamin C contained in orange juice enhance the immune system and also helps fight the disease-causing bacteria as tuberculosis.
Horsetail(*Equisetum arvense*)	a spoonful of horsetail juice on a daily basis	Reduce the deficiency of silica in tuberculosis patient.
Garlic(*Allium sativum*)	50 ml of concentrated garlic syrup twice a day	Work against microbes, harmful organisms, bacteria, fungi, parasites and viruses.
Herbal Tea	Licorice root tea prepared with only licorice roots	Use to get remedies from cough lung disease.
Propolis	Tea spoon trice daily	Immunostimulator.
Mint Juice(*Menta pepperita*)	A glass of fresh mint juice mixed with 150 ml of carrot juice, two spoons of honey and malt vinegar. The juice can be administered thrice everyday	Acts as a detoxifying agent and cleanses the body of all the anti-tuberculosis drug side effects.
Pineapple Juice	pepper and a dash of salt and honey can be administered to patients once everyday	This is found to be extremely helpful in dissolving mucus of the lungs in tuberculosis.
American ginseng(*Pinax ginseng*)	Three times a day	Ginseng contains minerals and nutrients that help build immunity.
Vitamin Supplements	As per diet	Act as energy sources and also act as immunity enhancer.

Alkaloids

Many of the earliest isolated pure compounds with biological activity were alkaloids as they are easy to isolate. The nitrogen generally makes the compound basic and it is found in the salt form in plants. Alkaloids are extracted with the water and then regain in crystalline form by treating with base. Pure alkaloids are containing antimycobacterial activity [16]. The basic unit in the biogenesis of the true alkaloids are amino acids. Non nitrogen containing derived from terpene units and methionine is responsible for addition of methyl group in nitrogen atoms.

23 new and known naturally occurring alkaloids and analogs have been assayed and found to have antimycobacterial activities (table.3). All these alkaloids are extracted from different parts of plant like roots, rhizomes and they belong from carbazoles and indole alkaloid. Indoloquinoline alkaloid has significant activity against *M. fortuitum* [19].

The benzoxazole alkaloids, marine metabolites, are also strong inhibitors of *mycobacterium tuberculosis*. They all contain oxazole moiety which also present in oxazolidinone having strong antitubercular activity [20].

Iminium salt demonstrates appreciable activity against *M. avium*, *M. Bovis*, BCG and *M. smegmatis*. It is believes that iminium ions increase the lipophilicity thence improve bioavailability of alkaloids [21].

Table 3: Antimycobacterial compounds by class, source, model used, and activity [23-36]

Compound Class	Sources	Activity MIC (µg/ml)
Alkaloids:		
3-Formylcarbazole	*Clausena excavate*	100
	C. excavate	50
3-Methoxycarbonylcarbazole	*C. excavate*	100
2-Hydroxy-3-formyl-7-methoxycarbazole	*C. excavate*	100
Clauszoline J	*Cryptolepis sanguinolenta*	16
Cryptolepine HCl	(ATCC6841)	12.5
Echinuline	BCG	169.9
Pseudopteroxazole	*Chaetomium globosum*	12.5
Seco-pseudopteroxazole	*Pseudopterogorgia elisabethae*	12
Homopseudopteroxazole	*P. elisabethae*	12.5
	P. elisabethae	
Sanguinarine	*Sanguinaria canadensis*	24.5
Flavonoids,Coumarins, Chromone, Chalcone:		
Flavonols	*Haplopappus sonoriensis*	–
Flavone	*Lysionotus pauciflorus*	–
Ostruthin	*Peucedanum ostruthium*	6.7
Licochalcone A	*Glycyrrhiza inflata*	7.1
Terpenoids:		
Erogorgiaene	*Pseudopterogorgia elisabethae*	12.5
Potamogetonin	*Potamogeton malaianus*	100
Phorbol ester	*Sapium indicum*	50
Phorbol ester	*S. indicum*	3.12
Phorbol ester	*S. indicum*	25
Steroids, Saponins:		
Stigmasterol	*M. citrifolia*	32
Epidioxysterol	*M. citrifolia*	2.5
Physalin B	*Physalis angulata*	>128
Jujubogenin	*analogue Colubrina retusa*	10
Peptides:		
Hirsutellide	*Hirsutella kobayasii*	6-12
Beauvericin	*Paecilomyces tenuipes*	12.5
Enniatin analogue	*V. hemipterigenum*	1.56
Syringomycin	*Pseudomonas syringae*	1.5

Flavones, coumarins, chromones and Chalcone

Flavones are biological natural products containing aromatic heterocyclic skeleton of flavan (2-Phenylbenzopyran) but no nitrogen in plants. Flavonoids are usually classified into main 6 subgroups as below plus flavans, neoflavonoids, flavonols, aurons, catechins according to the structural patterns.

- Flavonols (Hydroxy derivatives of flavones).
- Flavones (skeleton: 2-phenylchromen-4-one).
- Isoflavones (skeleton: 3-phenylchromen-4-one).

- Flavonones (derivation by reduction of the 2(3) C=C bond).
- Flavanols (derivation by reduction of the keto group):(+)-Catechin, (+)-Gallocatechin, (-)-Epicatechin (EC).
- Anthocyanidins (aglycones of the glycoside anthocyanins): Apigeninidin, Cyanidin.

Coumarin show the anti bacterial and anti fungal properties. Flavonols 24a–c isolated from Haplopappus sonoriensis (Asteraceae) and flavones 25 from Lysionotus pauciflorus (Gesneriaceae) the active principles of extracts from these plants. Leaf extract of *H. rigidus*, of species Haplopappusare use to treat cough and as antituberculosis agent. The coumarins used in anti bacterial. [26, 28, 35]

M. avium and *M. bovis* are strongly inhibited by licochalcone obtained from glycyrrhiza inflate with low MIC of 5-20 mg/ml and number spices are use as throat demulcents.

Terpenoids

Newly found that 118 synthetic and natural plant terpenoids to have moderate to high antimycobacterial activity against mycobacterium tuberculosis [36]. Secondary metabolites are very essential for the treatment of some antibacterial disease and terpenes are one of them and they are obtaining from natural plants [37]. We used low temperature chromatography technique to separate plant terpenes nearly a half of a century ago. The isoprene unit which can build upon in various ways is a five-carbon molecule [38].

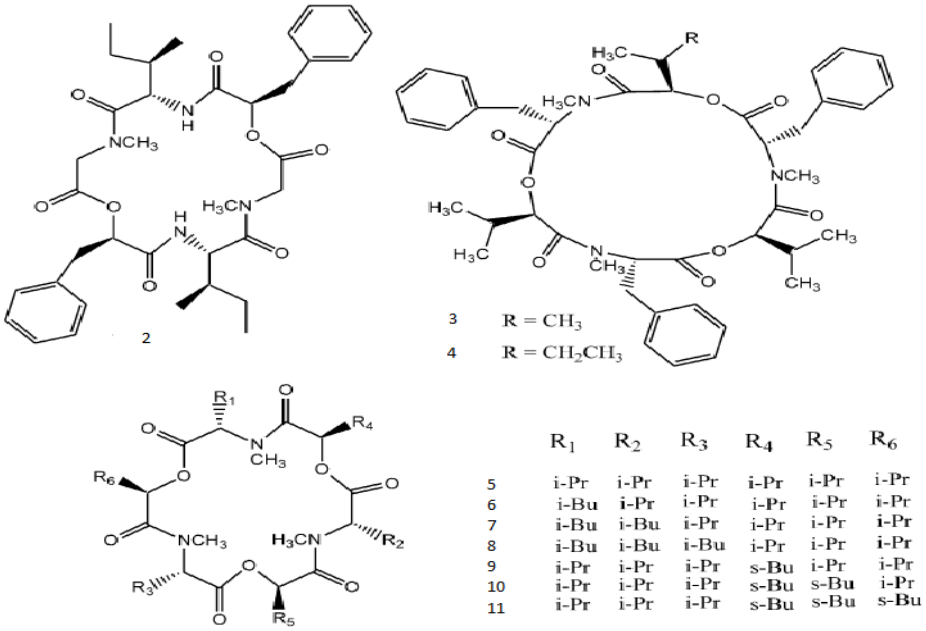
Isoprene unit bonded to second isoprene to form monoterpenes (C10), sesquiterpenes contain three unit of isoprene (C15) and diterpenes (C20) and triterpenes (C30) contain 2 and 3 isoprene units respectively. Some of the plants are evaluated in the table (2) from which the natural occurring terpenes have been isolated. Sapium indicum (Euphorbiaceae), Pseudopterogorgia elisabethae, marine sponge Smenospongia aurea, Croton kongensis
(Euphorbiaceae) Xanthocyparis nootkatensis (synonym Chamaecyparis nootkatensis) (Cupressaceae) all of these are reported to have activity against *mycobacterium tuberculosis* Constantine and other species of mycobacterium [39-41].

Steroids and saponins

The human use of *M. citrifolia* root decoctions as a medication to treat tuberculosis was reported [42].Some of compound of steroidal nature obtains from *Morinda citrifolia* (Rubiaceae) are use in antituberculosis treatment. The saponin extracted from the gorgonian octocoral Eunicea pinta, as well as the jujubogenin analogue from *Colubrina retusa* (Rhamnaceae), are also use in antituberculosis treatment, *Physalis angulata* (Solanaceae) are moderately active against mycobacterium tuberculosis. Some sterols from Ruprechtia triflora (Polygonaceae) with moderate to very good antimycobacterial activity against mycobacterium tuberculosis, MIC values range from 2 to 128 mg/ml [42].

Peptides

The cyclodepsipeptides in (fig 2) from fungi all have modest to high antituberculosis activity MIC ranging from 1.56 to 25 mg/ml[43,44].



	R_1	R_2	R_3	R_4	R_5	R_6
5	i-Pr	i-Pr	i-Pr	i-Pr	i-Pr	i-Pr
6	i-Bu	i-Pr	i-Pr	i-Pr	i-Pr	i-Pr
7	i-Bu	i-Bu	i-Pr	i-Pr	i-Pr	i-Pr
8	i-Bu	i-Bu	i-Bu	i-Pr	i-Pr	i-Pr
9	i-Pr	i-Pr	i-Pr	s-Bu	i-Pr	i-Pr
10	i-Pr	i-Pr	i-Pr	s-Bu	s-Bu	i-Pr
11	i-Pr	i-Pr	i-Pr	s-Bu	s-Bu	s-Bu

Figure no: 2- structure of Cyclodepsipeptides [45]

Other classes of compounds

Newly identified 13 natural product inhibitors of a novel detoxification enzyme, mycothiol-S-conjugate amidase (MCA) unique to the actinomycetes [15]. Compounds represent six different structural classes and founds IC50 (µM) ranging from 0.1 to 100.out of these four are found to have inhibitory action on mycobacterium tuberculosis. Newly peperine dimer extracted from Piper chaba (Piperaceae) also has antituberculosis activity with MIC of 12.5µg/ml.

Future Prospects

New herbal drug are developing containing different secondary metabolites for the treatment of tuberculosis. Current knowledge on natural herbal secondary metabolites can be utilized for development of new trends in herbal antituberculosis research. Polypeptides to proteins, all have efficient antituberculosis effect. Secretions from plants containing different metabolites show inhibition in the growth of mycobacterium tuberculii act as causative agent of tuberculosis. Flavones is thought to play a major role in the antioxidant activity associated with tuberculosis. The search for drugs that may inhibit and kills the bacteria, and thus improve treatment of tuberculosis patients, is considered as a frontier in the search for novel antituberculosis agents. Medicinal plants that have been shown to improve the tuberculosis state without producing any side effects in the patient lead to safe antituberculosis treatment.

CONCLUSION

Tuberculosis is a major threat to the health of million of populations in the developing and developed countries not only the *Mycobacterium tuberculosis* but also other species of mycobacterium are health concerns and new safe and herbal drugs are urgently required to counteract growing resistance towards currently available drugs.

A large number of plants are used in Asia and Africa to treat tuberculosis and related symptoms such as chronic coughs and respiratory complaints. For this 180 species that have been discovered as being employed for such purposes, around 30% of these have been investigated for antimycobacterial activity, as published in the available scientific literature. Most of these

investigations consist of *in vitro* tests with saprophytic and non-pathogenic *Mycobacterium* species.

It is shown that studies with useful lead extracts or isolated compounds related to different natural metabolites utilizing for pathogenic strains and *in vivo* systems need to be passed out to verify their antimycobacterial activity. Many selected family of plant kingdom are having Antituberculosis activities or have develop for antiTB agents, for example several plants of Asteraceae have been screened for antimycobacterial efficacy with encouraging results. There is no doubt that natural products, having specific chemical structures and powerful antimycobacterial effects are remain important participants in the development of new generations of antimycobacterial drugs.

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REFERENCES

[1] World Health Organization, Global Tuberculosis Control; Surveillance, Planning And Financing WHO, Geneva, Switzerland **2008**.
[2] R. J. O'Brien, P.P. Nunn, *Am .J Resp. Crit .care*, **2001**,162, 1055–1058.
[3] P. Sensi, G.G.Grassi, Chemotherapeutic agents. In: Abraham, D.J. (Ed.), Burger's Medicinal Chemistry and Drug Discovery, (John Wiley & Sons Inc., **2003)**, 821–824.
[4] J.D.McKinney, *Nat. Med.*, **2000,** 6, 1330–1333.
[5] E.M.Zager, R.McNerney, Multidrug-resistant tuberculosis. BioMedCentral Infectious Diseases 8, 10, **2008**, doi: 10.1186/1471-2334-8-10.
[6] E.L .Corbett, B.Marston, G.J.Churchyard, K.M.De Cock, Tuberculosis in sub-Saharan Africa: opportunities, challenges, and change in the era of antiretroviral treatment. Lancet 367, **2006**, 926–937.
[7] W.H.Lewis, *Econ. Bot*, **2003,** 57,126–134.
[8] W.H. Lewis, G.Lamas, A.Vaisberg, D.G. Corley, C. Sarasara, J.Alba´ n, R.Apanu´ , R.Castro, C.Crandall, M.P.F.Elvin-Lewis, W.Fritz, G.Hammond, P. Klueh, B. Milla´ n, A.L.Okunade, V.Pacheco, O.Tovar, G.Yarapaita´ n, *Pharmaceut. Biol*, **1999**, 37, 66–83.
[9] G.M.Cragg, D.J.Newman, K.M.Snader, *J. Nat. Prod*, **1997, 60**, 52–60.
[10] I.M.S.Eldeen, J.van Staden, *S. Afr. J. Bot.*, **2007,**73, 248– 251.
[11] E.M.Zager, R.McNerney, Multidrug-resistant tuberculosis. BioMedCentral Infectious Diseases 8, 10, **2008**,doi:10.1186/1471-2334-8-10
[12] World Health Organisation, Global Tuberculosis Control: Surveillance, Planning and Financing. WHO, Geneva, **2008**, www.sahealthinfo.org/ noveldrug/ novelpamphlet.html(accessed 30 April 2008)
[13] M. Sanjappa, Plant diversity in India—status, conservation and challenges (P. Maheshwari Medal Award Lecture). In: XXVIII Conference of Indian Botanical Society, Oct. 24–26, **2005**, 5–6.
[14] S.M.Newton, C.Lau, S.S.Gurcha, G.S.Besra, C.W.Wright, *J. Ethnopharmacol*, **2002,** 79, 57–67.
[15] Nancy A. Knechel, *Crit Care Nurse* **2009**, 29, 34-43.
[16] S.M Newton, C. Lau, C.W.Wright, *Phytother*, **2000**, Res. 14, 303–322.

[17] F Stickel, D Schuppan. Herbal medicine in the treatment of liver diseases. Digestive and Liver Disease. **2007**; 39:293–304. doi: 10.1016/j.dld.2006.11.004.
[18] Ho MJ. Perspectives on tuberculosis among traditional Chinese medical practitioners in New York City's Chinatown. Culture, Medicine & Psychiatry. **2006**; 30:105–22. doi: 10.1007/s11013-006-9010-6
[19] S.H.Gillespie, E.D.Morrisseyl, D.Everett, *J. Med. Microbiol*, **2001,** 50, 565–570.
[20] M.H. Cynamon, S.P.Klemens, C.A.Sharpe, S.Chase, *Antimicrob. Agents Chemother*, **1999,** 43, 1189–1191.
[21] N.Rastogi, J.Abaul, K.S.Goh, A.Devallois, E.Philogene, P.Bourgeois, *Immunol. Med. Microbiol*, **1998**, 20, 267–273.
[22] A.Sunthitikawinsakul , N. Kongkathip, B. Kongkathip, S. Phonnakhu, S. Phonnakhu, J.W. Daly, T.F.Spande, Y.Nimit, S. Rochanaruangrai, *Planta Med*, **2003**, 69, 155–157.
[23] S.Gibbons, F. Fallah, C.W.Wright, *Phytother. Res*, **2003**, 17, 434 43.
[24] S.Kanokmedhakul, K. Kanokmedhakul, N. Phonkerd, K. Soytong,P. Kongsaeree, A. Suksamrarn, *Planta Med*, **2002**, 68, 834–836.
[25] A.D.Rodrı´guez, C. Ramı´rez, *J. Nat. Prod*, **2001**, 64, 100–102.
[26] J.I.Murillo, R.Encarnacion-Dimayuga, J.Malmstrom, C.Christophersen, S.G. Franzblau, *Fitoterapia* **2003,**74, 226–230.
[27] P.Kittakoop, S. Wanasith, P. Watts, J. Kramyu, M. Tanticharoen, Y. Thebtaranonth,. *J. Nat. Prod*, **2001**, 64, 385–388.
[28] Y.Xu, Z.-Bi. Hu, S.-C. Feng, G.-J.Fan,. Yaoxue Xuebao ,**1979**, 14, 447–448.
[29] P.Chumkaew, C.Karalai, C.Ponglimanont, K.Chantrapromma, *J. Nat. Prod*, **2003**, 66, 540–543.
[30] A.H. Janua´ rio, E.R. Filho, R.C.L. Pietro, S.Kashima, D.N. Sato, S.C.Franc¸, *Phytother. Res*, **2002**, 16, 445–448.
[31] H.N. ElSohly, S. Danner, X.C. Li, A.C. Nimrod, A.M. Clark, *J. Nat. Prod*,**1999**,62, 1341–1342.
[32] N.Vongvanich, P.Kittakoop, M.Isaka, S.Trakulnaleamsai, S.Vimuttipog, M.Tanticharoen, Y.Thebtaranonth, Hirsutellide A, *J. Nat. Prod*, **2002**,65, 1346–1348.
[33] C.Nilanonta, M.Isaka, P. Kittakoop, P.Palittapongarnpim, S.Kamchonwongpaisan, D.Pittayakhajonwut, M.Tanticharoen, Y .Thebtaranonth, *Planta Med*, **2000**, 66, 756–75.
[34] E.Buber, A.Stindl, N.L.Acan, T.Kocagoz, R.Zocher,*Nat. Prod. Lett*, **2002**, 16, 419–423.
[35] A. Schinkovitz, S. Gibbons, M. Stavri, M.J. Cocksedge, F.Bucar, Ostruthin*: Planta Med*, **2003**, 69, 369–371.
[36] C.L.Cantrell, S.G.Franzblau, N.H.Fischer, *Planta Med*, **2001**, 67, 685–694.
[37] MF. Balandrin, JA. Klocke, ES. Wurtele, W.H Bollinger. *Science.* **1985**, 228, 1154-1160.
[38] Clements RL, *Science*,(**1958**), 128: 899-900.
[39] J. Thongtan, P. Kittakoop, N. Ruangrungsi, J. Saenboonrueng, Y. Thebtaranonth, *J. Nat. Prod*, **2003**,66, 868–870.
[40] G.H., J.J.Karchesy, S.G. Franzblau, L.E.LaFleur, (+)-Totarol from Chamaecyparis nootkatensis and activity against Mycobacterium tuberculosis. *Fitoterapia* , **2001**, 72, 572–574.
[41] I. Muhammad, J.S. Mossa, M.A. Al-Yahya, A.F. Ramadan, F.S. El-Feraly, *Phytother. Res*, **1995**, 9, 584–588.
[42] Okabe, M., **1940**. Investigation of the medicinal plants found on the Palau Islands, their virtues and popular remedies. Bull. Trop. Indus. Palau, South Sea Islands, Japan. no. 5. [Japanese].
[43] G.M.Woldemichael, S.G.Franzblau, F.Zhang, *Planta Med*, **2003**,69, 628–631.
[44] C.J Jackson, D.C. Lamb, D.E. Kelley, S.L. Steven, *Microbiol. Lett*, **2000**,192, 159–162.
[45] L. Okunade Adewole , *Phytochemistry*,**2004**, 65, 1017–1032.