

Phytomedicinal Investigation from Six Mangrove Species, North Sumatra, Indonesia

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Abstrak

Investigasi Fitomedisinal Enam Spesies Mangrove, Sumatera Utara, Indonesia

Mangrove adalah tanaman yang toleran terhadap garam dan dikenal kaya sebagai sumber metabolit sekunder dengan potensi bahan obat alami seperti triterpenoida, alkaloida dan fitosterol. Zat kimia ini merupakan senyawa aktif untuk pengembangan agen bioaktif baru. Investigasi fitomedicinal terhadap daun dan akar dari enam spesies mangrove di Sumatera Utara, Indonesia, yaitu *Acanthus ilicifolius*, *Bruguiera parviflora*, *Ceriops tagal*, *Rhizophora apiculata*, *Sonneratia caseolaris* dan *Xylocarpus granatum* dianalisis untuk karakterisasi profil kimianya. Identifikasi struktur fitokimia diverifikasi dengan perbandingan waktu retensi pada kolom GC dengan standar otentik dan interpretasi spektrum GC-MS. Skrining fitokimia menghasilkan pentasiklik triterpenoida dari golongan lupane, oleanane, ursane, kolesterol, fitol, squalene dan fitosterol. Triterpenoida dan fitosterol merupakan proporsi terbesar dari senyawa yang diisolasi. Komponen triterpenoida dan fitosterol masing-masing terdiri 7 dan 4 senyawa. Komponen utama dari triterpenoida adalah lupeol, α -amyirin, β -amyirin dan taraxerol. Fitosterol utama terdiri dari β -sitosterol, campesterol dan stigmasterol. Data ini dapat berkontribusi sebagai sumber bahan baku untuk pengembangan konstituen fitomedicinal dan agrokimia dari tanaman mangrove.

Kata kunci: bioaktif, β -sitosterol, lupeol, mangrove, fitokimia, fitomedicinal, fitosterol, triterpenoid

Abstract

Mangroves are salt tolerant plant and rich sources of secondary metabolites with potential medicinal value such as triterpenoids, alkaloids and phytosterols. These chemicals are promising active compounds for the development of novel bioactive agents. Phytomedicinal investigation of the fresh leaves and roots from six mangrove species, namely *Acanthus ilicifolius*, *Bruguiera parviflora*, *Ceriops tagal*, *Rhizophora apiculata*, *Sonneratia caseolaris* and *Xylocarpus granatum* from North Sumatra, Indonesia were analyzed to characterize their chemical profile. Identifications of the phytochemical structures were verified by comparison of their retention time on the GC column with those of authentic standards and on the interpretation of GC-MS spectra. Phytochemical screening yielded pentacyclic triterpenoids of lupane, oleanane and ursane group, cholesterol, phytol, squalene and phytosterols. Triterpenoids and phytosterols comprised the major proportion of compounds isolated. The triterpenoids and phytosterols consisted of 7 and 4 compounds, respectively. The major components of triterpenoids were lupeol, α -amyirin, β -amyirin and taraxerol. The main phytosterols consist of β -sitosterol, campesterol and stigmasterol. These data are likely to contributing a raw material source for development of phytomedicinal and agrochemical constituents from mangroves.

Keywords: bioactive compound, β -sitosterol, lupeol, mangrove, phytochemical, phytomedicinal, phytosterol, triterpenoid

Introduction

Mangrove forests are widespread in the inter-tidal zone of tropical and subtropical regions, and are known as a source of natural products with

unique bioactivity. Indonesia is one of the world's great mangrove nations, cover over 300,000 square kilometers, which is 22.6 % of the global total (Giri et al., 2011). Mangrove plants produce various secondary metabolites, which is useful in its

interaction with the environment, various environmental stresses as well as in the development of the resistance against external attack (Sudha and Ravishankar, 2002). Plant secondary metabolites represent a vast resource of complex molecules valued and exploited for their pharmacological and other properties (Oksman-Caldentey and Inze, 2004). Because of their wide range of biological activities, isoprenoids that include triterpenoids and phytosterols are regarded as important as potential natural sources for medicinal compounds (Sparg *et al.*, 2004) and mangrove plants have long been used in traditional medicine to treat disease (Bandaranayake, 1998). Furthermore, a number of biological activities such as antiviral, antioxidant, anticancer, antimicrobial, and many other agents from different mangrove plants have been reported for triterpenoids and phytosterols (Bandaranayake, 2002; Wu *et al.*, 2008; Patra and Thatoi, 2011). These studies suggest that the mangrove tree species may become a potential source of natural medicinal compounds, which may open up another possibility of mangrove utilization.

Mangroves are also unique in these halophyte plants have cellular mechanisms to be tolerant with the high salinity, and have mechanism to take up water despite strong osmotic potentials (Tomlinson, 1986). Our previous studies shed some light on the triterpenoid content and gene expression of triterpenoid synthase in salt-adapted mangrove plants (Oku *et al.*, 2003; Basyuni *et al.*, 2009, 2011, 2012a, 2012b). These results suggested that triterpenoids function as self-protecting barrier against the external salt stress. In spite of the importance of triterpenoids and phytosterols as potential sources of chemical constituents and

pharmaceutical development, little information on the quantitative of triterpenoids and phytosterols distribution for North Sumatran mangrove has previously been available.

Therefore, detailed analyses of nonsaponifiable lipid of North Sumatran mangroves with the special reference to triterpenoids and phytosterols composition were required to provide some insight into the importance of mangrove utilization as a source of novel phytomedicinal and biologically active compounds. NSL basically represent simple lipid fractions except for fatty acids (saponifiable lipids) after alkaline hydrolysis of the total lipids, and contain sterols, long-chain alcohols and alkanes. Thus, these observations in concert prompted us to report triterpenoids and phytosterols distribution of the leaves and roots of six North Sumatran mangroves, *Acanthus ilicifolius*, *Bruguiera parviflora*, *Ceriops tagal*, *Rhizophora apiculata*, *Sonneratia caseolaris* and *Xylocarpus granatum* for the first time.

Materials and Methods

Sample collection

Six mangrove species commonly thriving in Pulau Sembilan, North Sumatra, Indonesia were collected: *Acanthus ilicifolius* L. (Acanthaceae), *Bruguiera parviflora* (Roxb.) Wight and Arn. ex Griffith (Rhizophoraceae), *Ceriops tagal* (Perr.) C.B. (Rhizophoraceae), *Rhizophora apiculata* Bl. (Rhizophoraceae), *Sonneratia caseolaris* (L.) Engl. (Sonneratiaceae) and *Xylocarpus granatum* K.D. Koen. (Meliaceae).

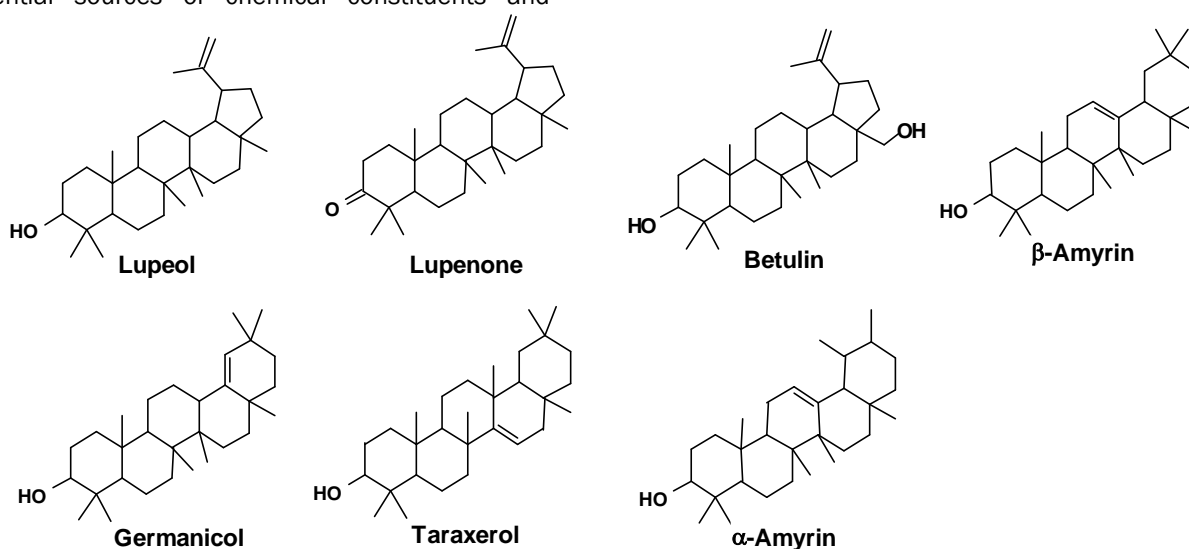


Figure 1. Structure of triterpenoids isolated from six mangrove leaves and roots. Identifications of triterpenoids were based on GC and GC-MS analysis as described in the method.

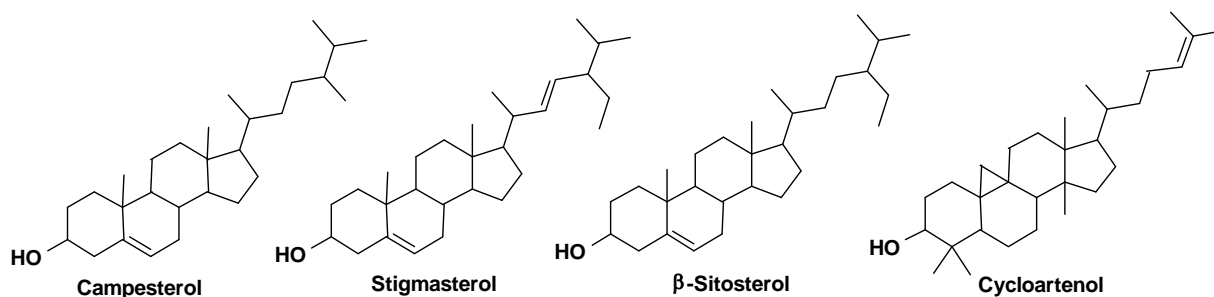


Figure 2. Structure of phytosterols isolated from six mangrove leaves and roots. Identifications of the phytosterols structures GC-MS analysis as described in the method.

Table 1. Total lipid and NSL contents of five species of mangrove leaves and roots

Species	Tissue	Total Lipid/tissue (mg/g)	NSL/tissue (mg/g)	NSL/Total Lipid (mg/g)
<i>Acanthus ilicifolius</i>	Leaves	1,55	0,07	0,04
	Roots	1,01	0,07	0,07
<i>Bruguiera parviflora</i>	Leaves	7,85	0,53	0,07
	Roots	0,65	0,37	0,58
<i>Ceriops tagal</i>	Leaves	3,20	0,69	0,22
	Roots	1,45	0,03	0,02
<i>Rhizophora apiculata</i>	Leaves	1,76	0,02	0,01
	Roots	1,25	0,44	0,35
<i>Sonneratia caseolaris</i>	Leaves	2,41	0,05	0,02
	Roots	1,11	0,11	0,10
<i>Xylocarpus granatum</i>	Leaves	3,19	0,32	0,10
	Roots	1,78	0,33	0,18

Lipid extraction

The leaves or roots (4 to 5 g in wet weight, respectively) were first grinded in liquid nitrogen, and extracted with 25 times volume of chloroform-methanol (2:1 by volume) (CM21). The cell wall debris insoluble to CM21 was removed by filtration through No. 2 filter paper (Advantec, Tokyo, Japan), and the extract was partially purified for lipid analysis as described previously (Basyuni *et al.*, 2009). The extract was concentrated to dryness, and the lipid weight was measured gravimetrically. Total lipid content was expressed as tissue weight (mg.g⁻¹ tissue).

Analysis of nonsaponifiable lipid (NSL)

The lipid extract containing 1 mg of total lipid was concentrated to dryness with nitrogen stream, saponified at 60°C overnight with 3% KOH in 94% ethanol. The NSL were partitioned into hexane by vigorous mixing, and basically contained sterols, alcohols and alkanes. The NSL were analyzed by gas chromatograph (GC 2014, Shimadzu, Kyoto, Japan) or gas chromatograph-mass spectrometer (GC-MS QP 2010, Shimadzu). An Rtx-1 (0.25 mm ID × 15 m, Restek, Bellefonte, PA, USA) column was used, and the column temperature was programmed as isothermal 300°C for 15 min. The carrier gas was helium with a flow rate of 0.70 ml.min⁻¹ (20 cm.s⁻¹), the temperatures for the injector and detector were 300°C and 300°C, respectively. For the

measurement of NSL content, approximately 1 mg of total lipid was saponified in 2 ml of 20% KOH (in 50% ethanol) at 90 °C for 10 min with reflux. NSL was extracted into 2 ml of hexane by vigorous shaking, and its weight was measured gravimetrically. NSL content was also expressed on the basis of fresh tissue weight (mg.g⁻¹ tissue) or total lipid weight (mg.mg⁻¹ total lipid).

Identification of chemical structure

The chemical structures of NSL were mainly identified by comparison of their retention time on GC column with those of authentic standards, and by interpretation of the mass spectrum. Authentic standards of b-amyrin, a-amyrin, lupeol, lupenone and cycloartenol were purchased from Extrasynthese (Genay, France). b-Sitosterol, stigmasterol and campesterol were purchased from Tama Biochemical (Tokyo, Japan), cholesterol from Wako Pure Chemical Industries (Osaka, Japan). Identifications of others (phytol and squalene) were only based on the similarities of their mass spectra to those in the mass spectra library (NIST 147 and 27, Shimadzu). The similarity indices were greater than 0.8 for these compounds. Ionization of sample was by electron impact (EI) at 70 eV for estimation of chemical structure, or by chemical ionization with methane as a reaction gas for the determination of molecular weight. The similarity search of the spectrum was carried out with the mass-spectrum library (NIST 147 and 27).

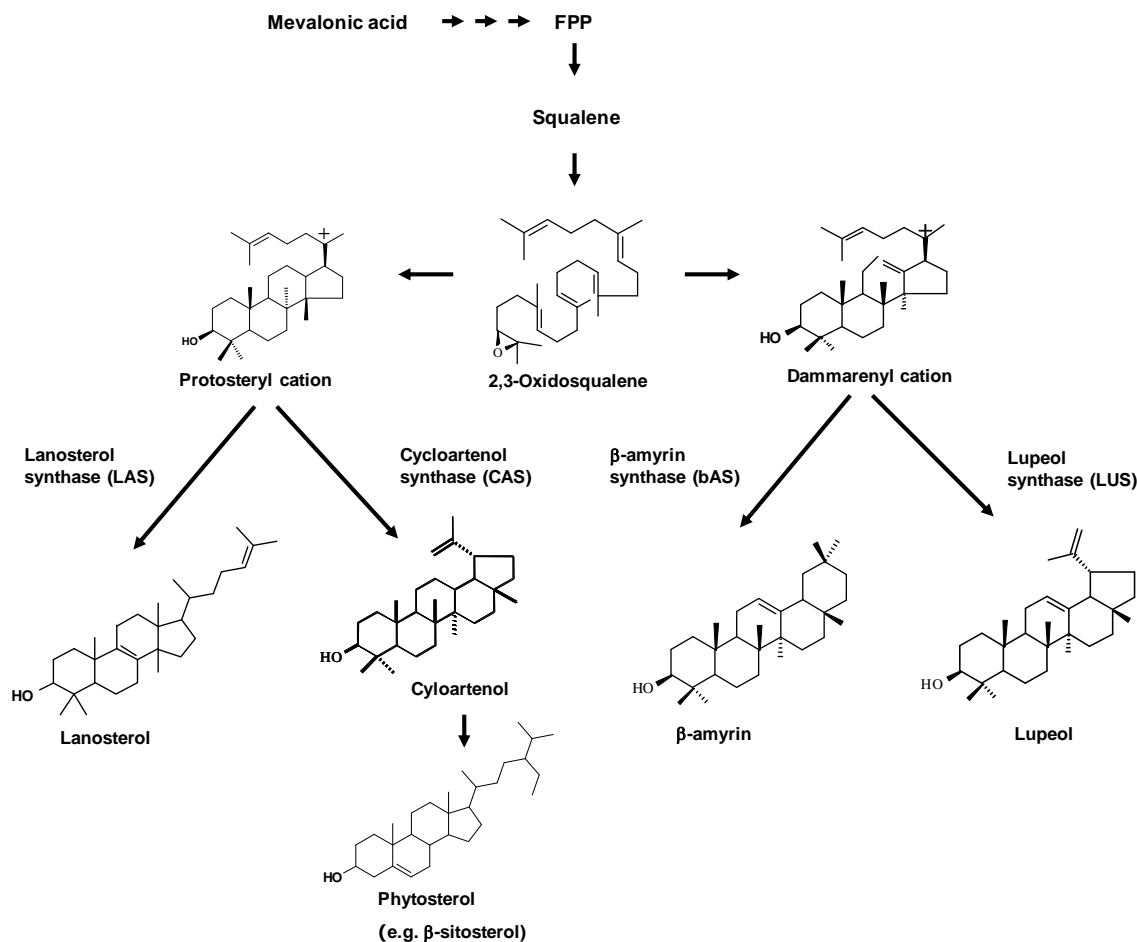


Figure 3. A Schematic of biosynthetic pathway of triterpenoids and phytosterols widely distributed in mangrove plants are biosynthesized from a common precursor 2,3-oxidosqualene by the enzyme oxidosqualene cyclases (OSCs). Enzymatic cyclization of 2,3-oxidosqualene into sterols proceeds via the protosteryl cation, which is then converted to cycloartenol or lanosterol. Triterpenoid synthesis, on the other hand, involves cyclization of the dammarenyl cation to produce a variety of triterpenoid.

Results and Discussion

The present study described the phytochemical distribution of six mangrove leaves and roots existed in North Sumatra, Indonesia. Special emphasis was on the triterpenoid and phytosterol compositions because these compounds were useful as biologically active medicinal components from mangroves. The chemical structures for triterpenoids identified in the six mangrove leaves and roots, *A. ilicifolius*, *B. parviflora*, *C. tagal*, *R. apiculata*, *S. caseolaris*, and *X. granatum* are presented in Figure 1. The pentacyclic triterpenoids largely are derived from three types of skeletons: lupanes (lupeol, lupenone, betulin); oleananes (β -amyrin, germanicol, taraxerol); an ursane (α -amyrin). α -amyrin, β -amyrin, lupeol, lupenone and betulin were identified by comparison of their retention time on GC column and mass-spectra with those of authentic standards. Identifications of chemical structures for germanicol

and taraxerol were accomplished by comparing their retention times and MS spectra with those in our previous report (Basyuni *et al.*, 2007a).

Figure 2 illustrates the chemical structures of phytosterol isolated from six mangroves leaves and roots. Campesterol, stigmasterol, β -sitosterol and cycloartenol were identified by authentic references as described above, comparison of retention time on GC analysis and mass spectra. Table 1 shows the total and NSL content of six species mangrove leaves. Total lipid content of the mangrove leaves ranged from 1.6 to 7.8 mg.g⁻¹ tissues with average of 3.3 mg.g⁻¹. The average was almost a one-quarter and a one-half of those reported for mangroves of India, 11.9 mg.g⁻¹ and Okinawan mangroves, 6.0 mg.g⁻¹, respectively (Ghosh *et al.*, 1985; Basyuni *et al.*, 2007b). Lipid content in the leaves for *A. ilicifolius* was slightly lower than the other 5 species. NSL content in the leaves was the lowest for *A. ilicifolius*, and the

highest for *C. tagal*. NSL comprised 1 to 22% of total lipids, and the average for 6 species was 8%. The lipid content of mangrove roots was lower than mangrove leaves with average of 1.2 mg.g⁻¹ (Tabel 1). *B. parviflora* had the lowest lipid content in the roots. On the other hand, NSL content in the roots was the lowest for *C. tagal*, and the highest for *R. apiculata*. NSL in the roots comprised 2 to 58% of total lipids, and the average for 6 species was 22%.

Table 2 and 3 summarize the triterpenoids, cholesterol, phytol, squalene and phytosterols contents of six mangrove leaves. Diversity compound was notable in the composition of phytochemical identified. Triterpenoids were the largest constituent for *A. ilicifolius*, *C. tagal*, *S. caseolaris*, and *X. granatum* (>50%). It was also noteworthy that the lupeol was predominant content of *C. tagal* leaves (79.1%). In contrast, to these triterpenoids-rich species, phytosterols were rather dominant in the species of *B. parviflora* (53.1%) with the major content of b-sitosterol (20.6%) and campesterol (20.5%). Phytol probably resulted from chlorophyll was present in NSL fractions of *C. tagal*, *R. apiculata* and *X. granatum*. Squalene, a precursor in the isoprenoid biosynthesis, was also detectable with appreciable quantities in all species.

The phytochemical contents of mangrove roots consisting of triterpenoids, squalene and phytosterols were listed in Table 4 and Table 5. Diversity compound with the species was also notable in the phytochemical composition of root. As was the cases for the leaves, triterpenoids and phytosterols comprised the main proportion in the roots. It is interesting to note that triterpenoids were predominantly present in six mangrove species (62.7%). The root of *R. apiculata* and *S. caseolaris* showed a higher concentration of b-sitosterol compared with other species. This result suggested that the acyl-transferase of mangrove may prefer triterpenoid alcohols rather than phytosterols as the esterification substrate (Oku *et al.*, 2003). Major six species distributed on Pulau Sembilan, North Sumatra were analyzed for their triterpenoid and phytosterol composition. The triterpenoid distribution showed variation with the mangrove species (Tables 2 and 3). Important variations in some of triterpenoid compounds suggested both inter- and intra specific variation. This observation is well in agreement with the previous results that the leaves of mangroves are chemotaxonomically distinguishable on the basis of their marker component (Hogg and Gillan, 1984; Ghosh *et al.*, 1985; Wu *et al.*, 2008; Basyuni *et al.*, 2012c). The diversity in the NSL distribution has been noted with mangrove species for both leaves and roots, implying the occurrence of divergent enzyme systems for isoprenoid biosynthesis. Despite diversity in the

carbon skeleton, all triterpenoids and phytosterols are biosynthesized from a common precursor substrate 2,3-oxidosqualene via oxidosqualene cyclases (OSCs) (Figure 3). 2,3-Oxidosqualene therefore locates at the branching point of isoprenoid pathway toward phytosterols or triterpenes biosynthesis (Abe *et al.*, 1993). The cyclization of oxidosqualene into triterpenoids and phytosterols are one of the most fascinating reactions found in nature and their biosynthetic pathway has been shown to be complicated and divergent (Figure 3). The enzymes, which perform these reactions, belong to OSCs family. In higher plants, OSC family member cycloartenol synthase and lanosterol synthase are responsible for sterol biosynthesis, and other OSCs are involved for triterpenoid synthesis.

Several biological activities have been demonstrated for triterpenoids: anti-inflammatory activity for taraxerol, a-amyrin, b-amyrin, lupeol and germanicol (Akihisa *et al.*, 1996; Singh *et al.*, 2002; Kim *et al.*, 2005); anti-carcinogenic activity for taraxerol and germanicol (Takasaki *et al.*, 1999; Jang *et al.*, 2004); insecticidal activity for taraxerol (Williams, 1999); anti-microbial, anti-protozoal, anti-proliferative, anti-invasive, anti-angiogenic and cholesterol lowering agent has been shown to lupeol (Gallo and Sarachine, 2009; Siddique and Saleem, 2011). Recently, it has been reported that pentacyclic triterpenes of the lupane, oleanane and ursane group as tools in cancer therapy (Laszczyk, 2009). Among these triterpene-type, oleananes and lupanes showed strong antitumor effect on kinds of cancers (Man *et al.*, 2010).

In this respect, mangrove species distributed in North Sumatra can be exploited as a source of medicinal compounds. For this reason, the mangrove tree species are potential medicinal plants, which may open up another possibility of mangrove utilization. It is however should be mentioned that *C. tagal* may be mainly valuable because of its abundance in lupeol as stated above, lupeol has several biological activities, and its abundance may be beneficial for the purification of this compound from the raw material. It is also noteworthy that triterpenoid abundant in the roots was scarcely detected in the leaves. This was almost true for all mangrove species, and suggested that the type of triterpenoid synthase in the root may differ from that in the leaf even in the same species. In this context, triterpenoid alcohols have been structurally distinguished from phytosterols, even though they share common biosynthetic pathway and hence the similar chemical structure (Figure 3). However, there has been no scientific rationale to distinguish functionally these compounds as the membrane structural lipid. The occurrence

Table 2. Triterpenoids contents (%) of six mangrove leaves

Species	Triterpenoids		Betulin	Germanicol	Lupenone	Lupeol	Taraxerol	Total
	α -amyirin	β -amyirin						
<i>A. ilicifolius</i>	11,2±0,1	9,2±2,3	nd	10,0±4,3	nd	10,2±1,7	11,9±3,4	52,5
<i>B. parvoflora</i>	8,8±3,2	2,9±1,5	7,5±3,8	2,9±1,9	3,5±1,7	6,7±4,3	8,7±3,7	41,0
<i>C. tagal</i>	nd	nd	nd	nd	nd	79,1±1,4	nd	79,1
<i>R. apiculata</i>	7,6±0,8	7,8±0,3	nd	3,7±0,0	nd	11,7±3,1	10,5±0,3	41,3
<i>S. caseolaris</i>	18,4±4,6	6,8±0	8,7±0,0	nd	nd	15,3±0,0	3,2±0,0	52,4
<i>X. granatum</i>	8,3±1,2	6,7±0,6	4,4±0,7	8,0±1,5	6,2±0,4	9,2±2,4	8,6±2,8	51,4

Data are represented as the mean of triplicate analyses ± SE, nd= not detected

Table 3. Cholesterol, phytol, squalene and phytosterols contents (%) of six mangrove leaves

Species	Cholesterol	Phytol	Squalene	Phytosterols	Campesterol	Cycloartenol	Stigmaterol	Total
				β -sitosterol				
<i>A. ilicifolius</i>	6,7±3,3	nd	4,7±1,6	12,5±0,6	8,9±2,7	7,0±0,9	9,5±2,9	36,1
<i>B. parvoflora</i>	3,3±0,8	nd	2,6±0,7	20,6±2,9	20,5±7,1	nd	12,0±7,6	53,1
<i>C. tagal</i>	nd	12,9±0,6	6,4±0,9	nd	nd	nd	1,6±0,0	1,6
<i>R. apiculata</i>	3,9±1,2	7,5±0	5,3±1,3	4,3±0,2	4,3±0,2	nd	2,6±0,4	42,0
<i>S. caseolaris</i>	8,4±1,8	nd	4,2±1,0	9,1±2,1	12,3±4,0	nd	13,6±3,8	35,0
<i>X. granatum</i>	7,5±1,0	7,1±4,1	5,9±2,4	7,7±1,9	7,2±1,7	6,5±0,3	6,6±0,2	28,0

Data are represented as the mean of triplicate analyses ± SE, nd= not detected

Table 4. Triterpenoids contents (%) of six mangrove roots

Species	Triterpenoids		Betulin	Germanicol	Lupenone	Lupeol	Taraxerol	Total
	α -amyirin	β -amyirin						
<i>A. ilicifolius</i>	17,7±4,2	13,2±2,3	8,7±1,9	5,7±0,5	4,4±1,0	13,6±2,0	8,6±1,3	72,0
<i>B. parvoflora</i>	3,0±0,9	12,0±1,9	21,9±4,5	8,8±2,1	9,8±4,3	7,1±0,06	9,5±4,1	72,0
<i>C. tagal</i>	14,9±1,4	12,1±1,5	7,8±2,5	6,8±1,5	7,7±1,0	10±3,0	10,9±2,8	70,2
<i>R. apiculata</i>	3,9±1,2	5,1±0,5	8,2±0,5	11,2±3,9	7,4±1,2	9,3±3,2	11,7±0,2	56,8
<i>S. caseolaris</i>	8,7±3,7	5,6±1,2	5,7±1,0	8,5±2,9	6,5±0,0	8,4±1,2	12,1±4,3	55,5
<i>X. granatum</i>	6,4±1,6	9,0±2,0	6,3±0,0	5,6±0,0	7,7±2,6	4,9±1,9	9,6±1,8	49,5

Data are represented as the mean of triplicate analyses ± SE, nd= not detected

Table 5. phytosterols contents (%) of six mangrove roots

Species	Squalene	Phytosterols	Campesterol	Cycloartenol	Stigmaterol	Total
		β -sitosterol				
<i>A. ilicifolius</i>	2,9±1,7	5,4±0,8	4,2±1,3	7,8±1,0	7,7±2,1	25,1
<i>B. parvoflora</i>	3,7±0,7	7,4±3,1	5,3±2,6	3,5±0,9	8,1±2,3	24,3
<i>C. tagal</i>	4,3±0,7	6,5±1,1	7,4±2,5	4,4±0,3	7,2±2,8	25,2
<i>R. apiculata</i>	3,5±1,3	21,7±3,9	5,5±2,5	nd	12,5±2,9	39,7
<i>S. caseolaris</i>	2,0±1,3	20,9±1,2	8,0±3,7	nd	13,8±0,2	42,5
<i>X. granatum</i>	4,1±1,6	11,2±0,2	17±0,4	5,0±0,9	13,2±3,9	46,4

Data are represented as the mean of triplicate analyses ± SE, nd= not detected

of squalene, an intermediate of isoprenoids biosynthesis, in the root may also support this view (Table 4 and Table 5).

Furthermore, a number of studies have been suggested that phytosterol to show anti-inflammatory, antibacterial, antifungal, and antitumor activities (Ling and Jones, 1995; Akihisa et al., 1996; Awad and Fink, 2000). For example, stigmaterol, a common plant steroid, relatively abundant present in *A. ilicifolius*, *B. parviflora*, *S. caseolaris*, *R. apiculata* and *X. granatum*, has been shown to have hypercholesterolemic effects (Bandaranayake, 2002). β -Sitosterol, the predominant phytosterol content in this study

particularly in the *B. parviflora* and *R. apiculata* leaves, *R. apiculata* and *S. caseolaris* roots have been reported to possess potent anti-inflammatory and antipyretic activities (Gupta et al., 1980). This finding suggested that phytosterols as well as triterpenoids are known as potent actives and highly characterized in mangrove plants, may become an ideal raw material for pharmaceutical development.

Conclusion

Our present data showed that triterpenoids and phytosterols as potential source to contribute medical value and agrochemical compounds,

especially found in *C. tagal*, because of its abundance in lupeol. It is also important that triterpenoid abundant in the roots was scarcely detected in the leaves. This was almost true for six mangrove species, and suggested that the type of triterpenoid in the root may differ from that in the leaf even in the same species, on the other hand, the triterpenoids in the root was biosynthesized in situ, not a translocation of the synthate from the leaves. The findings presented here require further studies may lead to phytopharmaceutical development. Exploration of the chemical constituent and other biological activities for triterpenoids promisingly potentiates the usefulness of mangrove plants.

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