ISSN 0975 - 6299 Vol 2 / Issue 3 / Jul - Sept 2011

International Journal of Pharma and Bio Sciences

RESEARCH ARTICLE PLANT BIOTECHNOLOGY

CHEMOSYSTEMATICS AND INDIGENOUS USES OF *SHOREA* IN SOUTH ASIA

KUNJANI JOSHI

Department of Botany, Patan Campus, Tribhuvan University, P.O. Box 2486, Kathmandu, Nepal

KUNJANI JOSHI
Department of Botany, Patan Campus, Tribhuvan University, P.O. Box 2486, Kathmandu, Nepal

***Corresponding author**

ABSTRACT

The Genus *Shorea* Roxb. ex Gaertn. (Dipterocarpaceae) is rich in species diversity with 194 species. In South Asia, the species of *Shorea* show great variation in morphological characters. These species are dominantly distributed in the forest habitats of the tropical and some parts of subtropical zones. Since more than two decades, the taxonomy of *Shorea* has been a point of discussion. Time to time, various taxonomists have tried to classify the species of *Shorea* on the basis of anatomical and morphological characters. This type of controvercy in the arrangement of the species can also be solved by the study of chemicals. Information regarding chemical constituents of the species of *Shorea* is very limited. Among the chemical constituents present in different parts of the plants, flavonoids are already proved as potentially important markers for taxonomic studies due to its characteristics such as structure variability, chemical stability, ubiquitous occurrence and easy and rapid identification. During the chemotaxonomic study of *Shorea* of South Asia, flavonoid aglycones (flavonol quercetin, myricetin, kaempferol, and flavones luteolin, apigenin and proanthocyanidin) and four flavonoid glycosides (quercetin 3-glucoside, quercetin 3-rutinoside, kaempferol 3,5-glucoside, and luteolin 7-glucoside) were isolated from the leaves of *Shorea* using standard procedures after separation and purification by paper chromatography in several solvent systems. The isolated flavonoids can be used as chemotaxonomic markers and also as active ingredients of many medicines. The ethnobotanical study of the species of *Shorea* was also carried out. At present, the species of *Shorea* and their parts are under serious threat due to habitat destruction and over-exploitation. An integrated approach is, therefore, essential for the sustainable management of *Shorea*. Strategies for sustainable management of the species have also recommended.

KEYWORDS

flavonoids, quercetin, luteolin, proanthocyanidin, taxonomy, Dipterocarpaceae.

INTRODUCTION

The genus *Shorea* Roxb. ex Gaertn. (Dipterocarpaceae) is rich in species diversity and is distributed in the forests of the tropical and some parts of subtropical plain as well as in the mountainous region (Whitmore, 1979; Ashton, 1980; Khan 1984; Grierson and Long, 1987; Kostermans, 1992; Janardhanan, 1993; Dayanandan *et. al*., 1999; Press, Shrestha, and Sutton, 2000; Joshi, 2001). The taxonomy of these taxa has always been a point of discussion. Recently it was realised that the controversy relating to taxonomy of the species can be solved by studying chemical constituents and their chemical characters i.e. the presence or absence of different phenolic compounds like flavonoids and tannins in the plants for species delimitation, systematic arrangement of species and tracing phylogenetic relationship of plant species. Among the chemical constituents, flavonoids are already proved as potentially important markers for taxonomic studies due to its characterisitics such as structural variability, chemical stability, ubiquitous occurrence and easy and rapid identification (Markham, 1982; Harborne, 1984). Moreover, the flavonoids are also used to solve the problems of plant identification where flowering and fruit development does not occur frequently (Joshi, 2001).

The information on the chemical constituents of the species of *Shorea* is very limited. Previous sporadic works were mainly concentrated on the isolation and identification of some triterpenoids, steroids and phenolic compounds including some flavonoids (Bate-Smith and Whitemore, 1959; Bate-Smith, 1962; Bandarnayak *et al.,* 1975; Giannasi and Niklas, 1977; Hegnauer, 1982; Ratnayake and Senanayake, 1996; Joshi, 2001, 2003). The pioneer study on flavonoid aglycone patterns of the species of *Shorea* was carried out by Bate-Smith and Whitemore (1959), who had reported flavonoid aglycones, ellagic acid and some unidentified fluorescent compounds on 10 species of *Shorea* from Malayan. Bate-Smith (1962), Giannasi and Niklas (1977), and Hegnauer (1982), have reviewed the status and isolated some flavonoids in some species of *Shorea* distributed in Malayan. Similarly some flavonoid aglycones have been reported from 11 species of *Shorea* from Sri Lanka (Ratnayake and Senanayake, 1996). During the chemotaxonomic study of Dipterocarpaceae, Joshi (2001, 2003) has isolated and identified aglycones and glycosid flavonoids from the leaves of *Shorea* of Sri Lanka and Nepal.

The parts and products of the species of *Shorea* are economically important for timber, resin, poles, and firewood (Trimen, 1893-1903; Ashton, 1980; Wijeyaratne, 1982/83; Kostermans, 1992; Joshi and Joshi, 1999; Gunatilleke & Gunatilleke, 2000; Joshi, 2001, 2003). The comprehensive documentation of the indigenous knowledge and practices and uses of the species of *Shorea* is still inadequately known.

In this paper an attempt has been made to present the leaf flavonoids, which were isolated and identified from the leaf of species of *Shorea* of South Asia and uses of the various parts of S*horea* by local people.

MATERIALS AND METHODS

The following materials and methods were used in the investigation of flavonoids and documentation of ethnobotanical information.

Plant materials

In this study, both fresh as well as herbarium specimens of various species of *Shorea* were used for isolation and identification of flavonoids. The fresh leaves were collected from the various countries of South Asia (Delwala, Kurunegala District and Sinharaja forest of Ratnapura District, Sri Lanka; National Botanical Garden, Bangladesh, Botanic Garden, India, and Hetauda, Makwanpur, Nepal). The botanical identity of the plants was determined using relevant literatures. The authenticity of the samples was carefully checked against herbarium specimens from Royal Botanical Garden, Peradiniya, Sri Lanka and Botanical Herbarium of India, Bagladesh National Herbarium, Bangaladesh and National Herbarium, Nepal. Some herbarium materials were also used in the investigation. Voucher specimens were lodged in the Department of Botany, University of Colombo, Sri Lanka and Environment and Biodiversity Research Laboratory, SchEMS, Pokhara University, Nepal.

Extraction and Identification of flavonoids

Acid hydrolysis of the leaf material was carried out in 2N HCl at 100^0 C for 30 to 45 min. The aglycones were extracted into ethyl acetate, concentrated and applied on Whatman No. 1. chromatography paper and cellulose TLC (thin layer chromatography) plates for 1-D chromatography against authentic flavonol and flavone markers myricetin, quercetin, kaempferol and apigenin obtained from Biochemica Fluka, 1999 and luteolin marker obtained from the leaves of carrot (*Daucus carota)*. The four solvents used were : HOAc (50% aqueous acetic acid); BAW (n-butanol-ethanol-water, 4:1:5, top layer); Forestal (acetic acid-conc. HCl-water 3:3:10) and PhoH (phenol saturation with water). Aglycones were confirmed by their Rf values, colour in UV (360nm) with and without $NH_{3,}$ with the markers. When anthocyanidins were detected by the breakdown of proanthocyanidins (during acid hydrolysis), these were extracted into amyl alcohol, evaporated and redissolved in ethanolic HCl and subjected to the same chromatographic procedure using BAW, Forestal and Formic acid. The two marker compounds used, delphinidin and cyaniding, were obtained from the egg plant (*Solanum melongena*) as given in Harborne (1973).

Glycosides were separated and purified from direct 70 % EtOH extract by paper chromatography on Whatman 3 paper (Markham, 1982; Harborne, 1984). They were based on UV spectral, shift measurements, Rf comparisions, and hydrolysis to yield aglycone and sugars. Sugars were identified by co-chromatography against markers in three solvents system including toluene – pyridine – acetic acid – water (4:1:1:3) which gives a clear separation of glucose and galactose. Flavonoid quercetin 3-xylosylglucoside was further characterized by partial acid hydrolysis in order to confirm xylosylglucoside. Co-chromatography with authentic markers was carried out to confirm identification.

Cluster Analysis

In order to see the co-relation between the species, cluster analysis was also performed using chemical data based only on leaves without any reproductive parts.

Collection and documentation of Ethnobotanical Information

Ethnobotanical information was collected using various field techniques such as direct interview, discussion with local people and by direct observations on the way the useful parts of this species were being collected and used. The key informants were village elders, school teachers, traditional healers or "Jhankri"and women.

RESULTS

Distribution and Taxonomy of Shorea

Although the major phytogeographical distribution of the species of *Shorea* in forest habitats of the tropical plain areas, some species are also found in the hilly partrs of the South Asia. The *Shorea* shows high endemism with 16 endemic species (Table 1).

TABLE 1
Shorea species in South Asia

Species	Distribution	Endemic species
Shorea affinis	Sri Lanka	Endemic
Shorea assamica	India	-
Shorea congestiflora	Sri Lanka	Endemic
Shorea cordifolia	Sri Lanka	Endemic
Shorea disticha	Sri Lanka	Endemic
Shorea dyeri	Sri Lanka	Endemic
Shorea gardneri	Sri Lanka	Endemic
Shorea lissophylla	Sri Lanka	Endemic
*Shorea malida**	Sri Lanka	Endemic
Shorea megistophylla	Sri Lanka	Endemic
Shorea oblongifolia	Sri Lanka	Endemic
Shorea ovalifolia	Sri Lanka	Endemic
Shorea pallescens	Sri Lanka	Endemic
Shorea robusta	Bangladesh, Nepal, Bhutan, India	-
Shorea roxburghii	India	-
Shorea trapezifolia	Sri Lanka	Endemic
Shorea tumbuggaia .	India	Endemic
Shorea worthingtoni	Sri Lanka	Endemic
Shorea zeylanica	Sri Lanka	Endemic

*Source: Ashton (1980); Joshi (2001); Khan (1984); Janardhanan (1993); Kostermans, (1992). *new species reported by Kostermans (1992)*

The taxonomy of these species has been the subjects of several recent monographs and has always been a point of discussion. Taxonomy of the Srilankan *Shorea* is very controversial and some species are excluded in the respective taxa and some are placed in different taxa (Trimen, 1893-1903; Ashton 1980; Kostermans, 1992). Ashton (1980) has considered the genus *Shorea* as a large entity including three well defined sections (*Shorea, Anthoshoreae, Doona*) with 15 species, based on their morphological characters. Kostermans (1992) has made a revision and compiled the species of S*horea* found in Sri Lanka and recognized 6 species and reinstated *Doona* as a separate genera and had included *Isoptera* in *Shorea*. A new species named *S. hulanidda* was also reported. Ashton's and Kosterman's classifications are based on anatomical and morphological characteristics. These two classifications show controversy in generic and species boundaries.

Chemical constituents of Shorea

The information on the chemical constituents, especially of the flavonoid patterns of *Shorea* is very limited. Ratnayake and Senanayake (1996) have reported some aglycones from 11 species of *Shorea* fron Sri Lanka. Joshi (2001, 2003) has made contribution reporting the flavonoids in the 15 species of *Shorea* of Sri Lanka and in *Shorea robuste*. She has reported myricetin and four glycosides compounds (quercetin 3-glucoside, quercetin 3- rutinoside, kaempferol 3,5 - glucoside, luteolin 7-glucoside) first time in the species of *Shorea.*

The results of leaf flavonoid aglycone and glycosides survey of 16 species of *Shorea* are presented in Table 2. The analysis of flavonoid data revealed that all taxa, which were surveyed, show the presence of highly diverse flavonoid patterns consisting of flavonol and flavone aglycones, proanthocyanidins and flavonol and flavone glycosides. Among the detected flavonoid aglycones, flavonol quercetin and flavones luteolin were detected in all sixteen species of *Shorea* whereas flavonol myricetin, kaempferol, flavone apigenin are found only in 19 %, 31 % and 13 % of the total species studied (Table 2). Another distinctive occurrence is the presence of proanthocyanidins in the leaves of thirteen species of the genus *Shorea*. In context of glycosidic pattern, quercetin 3-glucoside and quercetin 3-rutinoside were detected in 15 species analysed, other glycosidic compounds like kaempferol 3, 5-glucoside and luteolin 7- glucoside are detected in 6 % and 25 % of the total species respectively (Table 2). But the isolation of these compounds was very difficult due to the presence of interfering compounds in high concentration.

Table 2
Flavonoid Patterns in the Species of Shorea.

S. No.	Scientific Name*	M	Q	K	L	A	D	C	1	2	3	4	5	6
1	Shorea affinis	+	+	-	+	-	-	-	+	+	-	-	-	-
2	*Shorea congestiflora*	-	+	-	+	-	+	+	+	+	-	-	+	-
3	*Shorea cordifolia*	-	+	+	+	+	+	+	+	+	-	-	+	-
4	*Shorea disticha*	-	+	-	+	-	-	+	+	+	-	-	-	-
5	*Shorea dyeri*	-	+	-	+	-	-	+	+	+	-	-	-	-
6	*Shorea gardneri*	+	+	-	+	+	-	+	+	+	-	-	+	-
7	Shorea lissophylla	-	+	-	+	-	-	+	+	+	-	-	-	-
8	*Shorea megistophylla*	-	+	+	+	-	-	+	+	+	-	-	-	-
9	*Shorea oblongifolia*	-	+	+	+	-	+	+	+	+	-	-	-	-
10	*Shorea ovalifolia*	-	+	+	+	-	+	+	+	+	-	-	-	-
11	*Shorea pallescens*	-	+	-	+	-	-	-	+	-	-	-	+	-
12	*Shorea robusta*	-	+	-	+	-	-	+	+	+	-	-	-	-
13	*Shorea stipularis*	-	+	-	+	-	-	+	+	+	-	-	-	-
14	*Shorea trapezifolia*	+	+	-	+	-	+	+	+	+	-	-	-	-
15	*Shorea worthingtoni*	-	+	-	+	-	-	-	-	+	-	-	-	-
16	*Shorea zeylanica*	-	+	+	+	-	-	+	+	+	-	+	-	-

Key: M =myricetin, Q= quercetin, K= kaempferol, L= luteolin, A= apigenin, D=delphinidin, C=cyanidin, 1= quercetin 3-glucoside, 2= quercetin 3- rutinoside, 3= quercetin 3-xylosylglucoside, 4= kaempferol 3,5 - glucoside, 5 = luteolin 7-glucoside, 6= apigenin 5- glucoside, + = detected, - = not detected,
** =Species used for analysis*

Cluter analysis

Cluster analysis based on leaf flavonoid (aglycone and proanthocyanidin) data indicates that there are grouping tendencies, however, there are considerable overlapping in grouping patterns of species according to their similarity (Fig. 1). Although these tendencies relating to the linkages and separation of the group are complicated, they are significant from the taxonomic point of view. Some outcome of the cluster analysis agree with the classification of Sri Lankan *Shorea* proposed by Ashton (1980) and some with Kostermans (1992) on the basis of morphological characters.

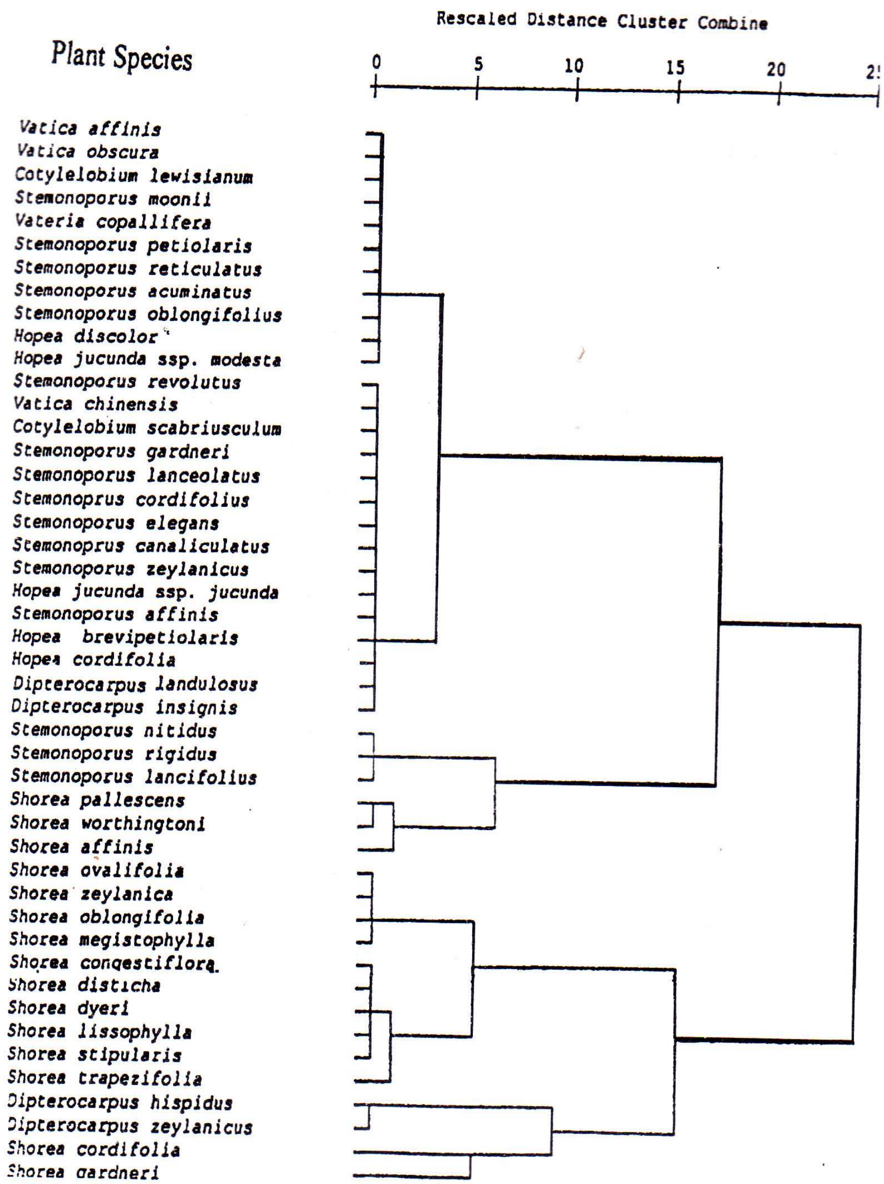

Fig. 1

Dendrogram derived by using chemometric data obtained from flavonoids of species of the family Dipterocarpaceae

Ethnobotanical value of Shorea

The people of South Asia traditionally use plant resources for their various basic needs such as food, firewood, timber, fodder, medicine and raw materials. Table 3 presents the useful parts and various uses of the different parts of the species of *Shorea* of South Asia. The rural people collect the useful plants or their products directly from the wild populations of the forest habitats which are already dwindling due to rapid deforestation, thus accelerating their genetic erosion. A recent survey on documented uses of plant products other than for timber has shown that 53 (23%) of a total of 211 tree species, both endemics and non-endemics, enumerated in the phytosociological study sites at Sinharaja, Sri Lanka are locally used for medicine, food, fuelwood and other domestic purposes (Gunatilleke, 1988). In the same way, *Shorea robusta* is extensively used by the rural communities of Nepal, India, Bangaladesh and Bhutan for many purposes (Table 3).

During the field survey, ethnobotanical information of *Shorea* was also collected. Various parts and the products of this specis are locally used for medicine, fuel, food and other domestic purposes i.e.

1. Timber: It is strong, elastic, and durable heartwood which is used for construction, doors, window frames, planking, carts, and carving etc. In the terai, timber is mainly used for construction of building, whereas it is the source of timber as well as fire wood in the villages of the hilly region..

2. Wood: it is used as firewood (with an energy content of about 22 700 KJ / kg for the heart wood, and 21 300 for the sap wood). It is also used to make charcoal.

3. Leaves: The leaves are lopped for fodder, though they are of only medium quality. They contain about 10% crude protein and the total digestible nutrients are 43 %.

4. Seeds: The seeds produce oil which is only used locally. After extraction of the oil, the cake is used to supplement cattle feed.

5. Others: The leaves are also widely used for temporary plates; flower is used to make curry; resin is used in making incense sticks which are used for worshipping, Resin is also used to cure dysentry and Gonorrhoea

Table 3

Species of Shorea with recorded uses in Sri Lanka, Nepal, Bangladesh, India and Bhutan

Scientific name and Local name	Distribution	Parts and Uses	Sources
Shorea affinis (Thw.) Ashton Beraliya Dun	Mixed forest below 1000m, wet zone, Sri Lanka	Wood: Plywood	Ashton (1980); Gunatilleke & Gunatilleke (2000); Joshi (2001)
Shorea congestiflora (Thw.) Ashton Tiniya	Lowland mixed forests 1000m, wet zone, Sri Lanka	Plywood	Ashton (1980); Joshi (2001)
Shorea cordifolia (Thw.) Ashton Kotikan Beraliya	Mixed forests on hills below 1000m, wet zone, Sri Lanka	Wood	Ashton (1980); Joshi (2001)
Shorea gardneri (Thw.) Ashton Koongili Maram; Rata Dun	Mid-mountain forest between 1000 and 1800m., wet zone, Sri Lanka	Red-brown hardwood used for construction and for railway sleepers	Ashton (1980); Joshi (2001)
Shorea megistophylla Ashton Honda-beraliya, Kana-beraliya	Mixed forests below 1000m., wet zone, Sri Lanka	Wood for plywood and light construction; Resin	Ashton (1980); Joshi (2001); Trlmen(1893-1903)

Shorea oblongifolia Thw. Pathuru, Yakahalu, Dummala	Wet parts upto 700 m, Sri Lanka	Timber; Resin used for varnishes	Ashton (1980); Joshi (2001); Kostermans (1992); Wijeyaratne(1982/83)
Shorea pallescens Ashton Ratu Dun	Mixed forests below 1000m ,wet zone, Sri Lanka	Timber	Ashton (1980); Joshi (2001); Trimen (1893 –1903)
***Shorea robusta* Gaertn.** Sal; Shakhuwa, Agrakha	Tropical parts of Nepal	Resin is used to cure dysentry and Gonorrhoea, is also employed as a substitute for pitch in caulking boats, and as an inferior quality of incense; Leaves: fodder, plate; Flower: to make curry; Wood: timber is used for railway sleppers and bridge construction.	Janardhanan (1993); Joshi (2001, 2003); Joshi & Joshi (1999); Khan (1984);
Shorea stipularis Thw. Nawadun, Nawada, Hulan-Idda	Wet, evergreen forest of lowland, up to 500 m, Sri Lanka	Bark is used for preventing the alcohol in palm wine to turn into vinegar	Ishwaran & Erdelen (1990); Joshi (2001); Kostermans (1992)
Shorea trapezifolia (Thw.) Ashton Yakahalu Dun	Hill forests between 600 and 1200m, wet zone, Sri Lanka	Wood for plywood; Fruits are made into flour and are largely consumed	Ashton (1980); Joshi (2001)
Shorea zeylanica (Thw.) Ashton Dun; Koongili	Mixed forests between 300 and 1400 m., wet zone, Sri Lanka	Timber	Ashton (1980); Joshi (2001)

DISCUSSION

One of the most significant present findings in the present investigation is the detection of flavonoids (flavonol quecetin, flavone luteolin, quercetin 3-glucoside, quecetin 3-rutinoside) in the species of *Shorea.* In respect to the presence of flavonoids in the species of *Shorea*, the results of the present flavonoid study agree with the result of Joshi (2001), who have reported aglycones: flavonol quercetin, flavone luteolin, and glycosides: quercetin 3-glucoside, quercetin 3-rutinoside, as the main common flavonoids in all Srilankan species of *Shorea* surveyed. These flavonoids can be regarded as taxonomic markers.

Another notable result of the present work is the absence of myricetin and presence of proanthocyanidin in the leaves of some species. From the taxonomic viewpoint, presence and absence of myricetin and proanthocyanidin character is very significant (Bate-Smith and Whitemore, 1959; Harborne, 1966). The presence of myricetin and proanthocyanidin is considered as a primitive character in dicots, particularly in woody plants (Bate Smith, 1962). This species can be regarded as advanced in flavonoid pattern because of the absence of myricetin. But, on the basis of presence of proanthocyanidin, this species show primitive flavonoid pattern.

The data also indicated that only flavonol glycosides were detected in the leaves of this species. As flavones are generally considered to be more 'advanced' than flavonols in evolutionary terms by loss of the extra 3-hydroxyl group (Williams *et.al.,* 1993), the presence of flavonols in this species indicates primitive flavonoid glycosidic patterns.

Though the characteristic features such as primitive and advance flavonoids might have important implications on the investigation of relationship between natural distribution of flavonoids and phylogenetic aspects of the various species of plants (Harborne, 1966; 1984), it is difficult to trace the linkages with other species due to the limited knowledge of the detected chemical constituents, especially flavonoids, in these species. More comprehensive studies including other aspects such as molecular, as well as on biogeographical areas are needed to arrive to the conclusion in this aspect.

Ethnobotanically, the contributions of the *Shorea* species are very significant to provide basic needs for the local communities. The rural people collect the useful parts or their products directly from the wild population of the forest habitats which are already dwindling due to rapid deforestation, thus accelerating their genetic erosion.

STRATEGIES FOR SUSTAINABLE MANAGEMENT

Some initiatives have already taken for the sustainable management of bioresources in South Asia, such as preparation of Biodiversity Conservation Policy, Strategy and Action Plan, National Conservation Strategy (NCS), Environmental Legislation, State of Environment Report. Despite the formulation of policy, strategies and plan and implementation of various activities for the conservation of the species and their habitats, there is a growing consensus among the conservationists that the conservation of bio-resources is entering into a stage of crisis, since there has been hardly any attempt to conserve these resources in an integrated manner (Joshi *et al*., 2003).Therefore, the following strategies should be implemented in order to conserve and sustainable use of the species of Dipterocarpaceae.

Formulation of Policy and Programme

In the present national policies and programmes, conservation and sustainable use of useful plants and their resources have not received high priority. Therefore, priority should be given to formulate national policy, action plan and programme related to management of the species and their habitats taking into consideration of the productivity of the species, needs of the people and sustainable use of resources. Programmes should also be initiated to identify and manage their critical habitats .

Documentation and Chemical Screening of the Species

Although investigation on the taxonomy and chemical constituents of Dipterocarpaceae has been conducted in different parts of the South Asia, there is a paucity of quantitative and systematic data on the status and biogeographical distribution, chemical constituents, habitat with ecological characteristics and possible vulnerability to environmental changes. Therefore, it is recommended that major thrust be given to an intensive inventory and documentation of species, chemical constituents, and uses of the species of *Shorea* and their habitats. However, the knowledge on chemical constituents, especially flavonoids, is limited and more comprehensive studies on other aspect e.g. molecular, cytological, ecology and biogeographical areas are also needed to provide more definite conclusion regarding the integrated classification. Though the various parts and the products are useful to fulfill basic needs of the people, proper documentation and study on the chemical constituents are essential before mass scale utilization.

Sustainable management of the species and their habitats and local people involvement

Human pressure in the species of *Shorea* is very immense. Moreover, the habitats of this species are also in degraded state due to over exploitation, unscientific management and unplanned landuse practices. Protection, and conservation of habitat and regeneration of forest would be a major positive contribution to enrich the growth of the species in nature. Hence, top priority should be given to *in-situ* conservation of this threatened species Such steps will not only contribute to protect the habitats but also help to maintain the ecological processes. Emphasis should also be given to conserve useful species in *ex-situ* conservation.It is also obvious that the success and sustainability of the conservation and sustainable management activities depend upon the involvement of the local people. Attempt should be made to launch special programmes for raising people's awareness about conservation and utilization of species.

ACKNOWLEDGEMENTS

The author is grateful to Dr. G. I. Seneviratne, Department of Botany, University of Colombo, for guidance, and to Professor K. Abeynayake, Dean, Faculty of Science and Professor R.L.C. Wijesundera, Head, Department of Botany, University of Colombo for giving the opportunity to carry out this research and encouragement. Thanks to Dr. Reene Grayer, Royal Botanical Kew Garden, UK for help and encouragement, Dr. Wijesundara, Director, Royal Botanical Garden, Peradeniya, Sri Lanka, and Mr. Upali Dhanasekera, Curator, National Herbarium, Paradeniya for confirming of the specimen identification and for providing plant materials. Profs. I. A. U.N. Gunatilleke and C. V. S. Gunatilleke, University of Peradiniya, Sri Lanka, Dr. B.P.M. Singhakumara, Department of Forestry and Environment, Jayawardenapura University for suggestion and providing some plant materials. Dr. S. K. Jain, Ex-Director, Botanical Survey of India, India, Prof. MD. S. Khan, Bangladesh and Dr. A.R.Joshi, Director, Academic Department, SchEMS, Pokhara University, Nepal for ecourgament.

REFERENCES

1. Ashton, P.S., Dipterocarpaceae. In: M. D. Dassanayake & F.R. Fosberg, (Eds), *A Revised Handbook to the Flora of Ceylon, Vol. I*, Amerind Publishing Co. Pvt. Ltd., New Delhi, India. pp 364-423, 1980.
2. Bandaranayake, W.M., Gunasekera, S.P., Karunanayake, S. Sotheeswaran, S. and Sultanbawa, M.U.S., Terpenes of Dipterocarpus and Doona species, *Phytochemistry* 14: 2043-2048, (1975).
3. Bate - Smith E.C., The phenolic constituets of plants and their taxonomic significance. *The Journal of the Linnaean Society of London* 58:95-173, (1962).
4. Bate-Smith E.C, and Whitmore, T. C., Chemistry and taxonomy in the Dipterocarpaceae, *Nature,* London, 184:795-796, (1959).
5. Dayanandan, S., Ashton P.S., Williams S.M., and Primark, R.B., Phylogeny of the Tropical tree family Dipterocarpaceae based on Nucleotide sequences of the chloroplast *rbc*L gene. *American Journal of Botany* 86: 1182-1190, (1999).
6. Giannasi, D.E. and Niklas, K.J., Phytochemistry, Pakarimoideae, Dipterocarpaceae of the Western Hemisphere, *Taxon* 26 (40): 341- 385 ((1977).
7. Grierson, A.J.C. and Long, D.G., *Flora of Bhutan including a record of plant from Sikkim,* Vol1, Part 2, Royal Botanic Gaeden, Edinburgh, 1987.
8. Gunatilleke, C.V.S., Trees of the Sinharaja forest and their non-timber products. In F.S.P. NG, ed., Trees & Mycorrhiza. Proceeding of the Asian Seminar 13-14 April, ,pp. 251-260, Forest Research Institute, Malaysia, 1988.
9. Gunawardana, Y.A.G.P., Sultanbawa, M.U. and Balasubramaniam, S., Distribution of some triterpenes and phenolic compounds in the extractives of endemic Dipterocarpaceae species of Sri Lanka, *Phytochemistry* 19:1099-1102, (1980}.
10. Harborne, J.B., The Evolution of Flavonoid Pigments in Plants, In : Swain, T. ed. *Comparative Phytochemistry*, Academic Press, London, pp.271-296, 1966.
11. Harborne, J. B., Flavonoids, In: Miller, L. P. (Ed), *Phytochemistry* Vol. I Van Nostrand Reinhold Company, New York. pp. 344-380, 1973.
12. Harborne, J.B., *Phytochemical Methods-A Guide to Modern Techniques of Plant Analysis*, 2nd Edition, Chapman and Hall, London, 1984.
13. Hegnauer, R., Phytochemistry of Dipterocarpaceae. In: P.S. Ashton, (Ed.), Dipterocarpaceae, *Flora Malesiana,* Ser 19 (2): 273 -274, (1982).
14. Ishwaran, N. and Erdelen, W., Conserving Sinharaja – An experiment in sustainable development in Sri Lanka, *Ambio* 19: 237-244, (1990).
15. Janardhanan, K.P. Dipterocarpaceae, In: B.D. Sharma and M. Sanjappa (Eds.), Flora of India, Vol.3. Botanical Survey of India, Calcutta, India, pp.206- 251, 1993.
16. Joshi, A. R. and Joshi, Kunjani, Kali Gandaki Watershed-Biodiversity, East Consult, Kathmandu, Nepal, 1999.
17. Joshi, Kunjani, *Chemotaxonomic Investigation on The Family Dipterocarpaceae Using Flavonoid Analysis*, Thesis submitted for the degree of Ph.D., University of Colombo, Sri Lanka, 2001.
18. Joshi, Kunjani, Senaviratne, I. and Senanayake, S. P., Leaf flavonoid aglycones pattern in the species of Dipterocarpaceae in Sri Lanka, *Biochemical Systematics and Ecology* 32: 329-336, (2004).
19. .Khan, M.S., Dipterocarpaceae, In: MD. S. Khan (Ed.),*Flora of Bangladesh*, No.25, Bangladesh National Herbarium,

Bangladesh Agricultural Research Council, Bangladesh, pp1-15, 1984.

20. Kostermans, A. J. G.H., *A Handbook of the Dipterocarpaceaew of Sri Lanka*, Wildlife Heritage Trust of Sri Lanka, Colombo, Sri Lanka, 1992.
21. Markham, K. R., *Techniques of Flavonoid Identification*, Academic Press, London, UK. 1982.
22. Press, J.R., Shrestha, K.K. and Sutton, D.A. , *Annotated Checklist of the Flowering Plants of Nepal*, The Natural History Museum, London and Central Department of Botany, Tribhuvan University, Nepal, 2000.
23. Ratnayake, R.M.C.S. and Senanayake, P., The occurrence of flavonoids in the genus *Shorea* in Sri Lanka, Abstract. In: *Fifty Second Annual Session Proceeding,* Sri Lanka Association for the Advancement of Science, Sri Lanka. pp. 106-107, 1996.
24. Trimen, H., *A Handbook to the Flora of Ceylon*, 5 Vols., Dulav Co., London, 1893-1903.
25. Whitmore, T.C.. Dipterocarpacea, In: H. Hara and L.H.J. Williams, (Eds). *An Enumeration of the Flowering Plants of Nepal,* (), British Museun (Natural History), London, UK, 1979.
26. Wijayaratne, R., A Botanist in Natural Products Research. *Phyta* 2 (20):46-48, (1982-83).
27. Williams C. A., Richardson, J. Greenham, J. and Eagles, J., Corelations between leaf flavonoids, taxonomy and plant geography in the genus *Disporum,* Phytochemistry 34 : 197-203, (1993).