Current Topics

Pharmacologically Active Constituents from Plants Used in Traditional Medicine

Prospecting for Bioactive Constituents from Traditional Medicinal Plants through Ethnobotanical Approaches

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Pharmacologically active constituents from traditional medicinal plants have received great attention as sources of novel agents, pharmaceutical intermediates, and chemical entities for synthetic or semisynthetic drugs due to their potent pharmacological activities, low toxicity, and economic viability. Numerous components have been isolated from traditional medicinal plants, including alkaloids, flavonoids, and terpenoids, and clinical and experimental studies suggested that these components have useful pharmacological properties such as antiinfectious, antioxidative, and antiinflammatory effects. In this review, modern ethnobotanical approaches to explore folk medicinal plants as candidates for drug discovery with the greatest possibility of success are discussed. Determining the bioactive mechanisms and tracing structure–activity relationships will promote the discovery of new drugs and pharmacological agents.

Key words bioactive constituent; traditional medicinal plant; antioxidative; antiinflammatory; drug discovery; modern ethnobotany

1. INTRODUCTION

As more people become aware of the potency and side effects of synthetic drugs, there is increasing interest in natural product remedies with diverse chemical structures and bioactivities against different diseases. Typically, many of these natural products are plant secondary metabolites (PSMs), and the major pharmacologically useful groups of PSMs can be divided into several categories that include alkaloids, flavones, terpenes, phenolics, and polyphenols[1,2] that have antiinfectious, antioxidative, and antiinflammatory effects. Twelve thousand plant-derived agents have been isolated in the past decades.[1] Approximately 30 plant-derived anticancer compounds were reported to be clinically active against various tumor types and used in clinical trials,[3] such as taxol, podophllotoxin, vinblastine, and others. PSMs are not only considered as direct sources of new pharmaceuticals but also provide unlimited opportunities for new drug leads because of their unmatched chemical diversity.[4]

The extensive research on different plant species and their therapeutic principles have led to a reevaluation of traditional medicine systems, including ayurveda, unani, and traditional Chinese medicine.[5,6] Many traditional medicinal plants (TMPs), which form part of traditional medicine, have the ability to synthesize plant-derived bioactive substances. In addition, TMPs have been used with varying success rates to cure and prevent disease throughout history. For these reasons, screening pharmacologically active ingredients from TMPs would be optimal in terms of efficiency, safety, and economics. Written records of medicinal plant use date back at least 5000 years to the Sumerians, and archeological records suggest even earlier use of medicinal plants.[7] Profound knowledge of medicinal plants in traditional cultures developed through trial and error over centuries, and the most important experiences were passed on verbally from one generation to the next.[6] Unfortunately, knowledge of TMPs has not reached a stable peak[8] but is eroding rapidly due to the disinterest of younger generations and the dying off of older generations who possess the knowledge.[9]

Ethnobotany plays an increasingly important role in preserving disappearing traditional knowledge, especially traditional medicinal knowledge. Ethnobotanical surveys to document the traditional uses of various indigenous plants not only recognize this undocumented knowledge but also provide new avenues for pharmacological investigations to improve healthcare for a range of ailments.[9,10] In addition, ethnobotanical information offers a viable alternative to high-throughput screening of bioactive substances.[11] For drug discovery, phytochemical and pharmacological research based on modern ethnobotany is considered a validating approach in the search for novel chemical entities and frameworks with potential as drug leads.[6,11,12] It is estimated that 122 drugs from 94 plant species have been discovered through ethnobotanical leads,[13,14] such as morphine, the main anesthetic alkaloid in opium, or vincristine, an antitumor compound.[15]

In addition to medicinal plants, folk practices are an important part of traditional medicine which can lead to pharmacologically active ingredients and new pharmaceutical discoveries. This review discusses pharmacologically active constituents from TMPs, including categories, pharmacologi-

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cal activity, and structure–activity relationships. We also describe the application of ethnobotany to bioactive ingredients or new drug discovery.

2. TMP-DERIVED ACTIVE CONSTITUENTS

Medicinal plants are rich sources of bioactive constituents or phytopharmaceuticals for the pharmaceutical industry and used in the production of a number of medicines.[16] Owing to their versatile applications, recently plant-derived substances have attracted great interest.[17] Plant-derived active constituents are mainly PSMs, and pharmaceutically significant PSMs or phytopharmaceuticals include alkaloids, terpenes, flavones, phenolics, and polyphenols[2,16,18–20] (Table 1).

2.1. Alkaloids Alkaloids are typical heterocylic nitrogen compounds biosynthesized from amino acids.[21] They can be classified in terms of their biological activity, chemical structure, or biosynthetic pathway.[18] Alkaloids are most commonly divided into five major groups depending on their biosynthetic amino acid (amino acid in parentheses)[18]: tropane, pyrrolidine, and pyrrolizide alkaloids (ornithine); benzylisoquinoline (tyrosine); indolequinoline (tryptophane); pyridine (pyridine); and quinolizidine and piperidine alkaloids (lysine).

After the first medically useful alkaloid, morphine, was isolated in 1805 from *Paver somniferum* (opium poppy), more plant-derived alkaloids have been found. For example, eight new pyrrolidinoindoline alkaloids (**1**–**8**) (Fig. 1) were isolated from *Selaginella moellendorfii*.[22] Over the decades, clinical and experimental studies have confirmed that many alkaloids from TMPs have anticancer, antiinflammatory, antiviral, antimicrobial, antioxidative, and acetylcholinesterase (AchE)-inhibitory activities, in addition to exerting immunomodulatory, hepatoprotective, and cardioprotective effects (Table 2).

2.2. Terpenoids The terpenes are one of the largest groups of PSMs and have the most diverse structures. Over 30000 different terpene structures have been reported.[18] The basic building block of terpenes, isoprene, is a 5-carbon molecule that is used to form more complex compounds. Based on the number of isoprenes, terpenoids can generally be divided into monoterpenoids (C10), sesquiterpenoids (C15), diterpenoids (C20), triterpenoids (C30), *etc*. From a pharmacological point of view, the important classes of terpenoids include sterols, essential oils, triterpenes, and saponins. These constituents possess a broad spectrum of pharmacological effects including antibacterial, antiviral, antifungal, antiinflammatory, anticancer, and antioxidant effects. The high medicinal and economic interest in them has focused on artemisinin, paclitaxel, gossypol, and zingiberene from TMPs in the terpenoid group.[18]

With the increased interest in traditional medicine, more studies on the phytochemistry and pharmacology of TMPs have been conducted, and many novel terpenoids have been discovered. Gutierrez-Lugo *et al.* isolated a new cycloartane-type triterpene from *Acalypha communis*, and the compound exhibited moderate antimicrobial activity against vancomycin-resistant enterococci.[30] A new secoiridoid glycoside, naresuanoside (**9**) (Fig. 2), isolated from stems of the Thai medicinal plant *Alstonia macrophylla* Wall. showed a significant inhibitory effect against AchE determined using the Ellman assay.[31] Two novel labdane diterpenes (**10**, **11**) (Fig. 2) isolated from the rhizomes of *Hedychium coronarium*, an important folk medicinal plant widely found in tropical and subtropical regions such as Japan, India, Brazil, South China, and Southeast Asia, showed moderate cytotoxicity against the A-549 (lung cancer), SK-N-SH (human neuroblastoma), MCF-7 (breast cancer), and HeLa (cervical cancer) cell lines.[32] Three new humulane-type sesquiterpenes (**12**, **13**, **14**) (Fig. 2) were isolated from *Pilea cavaleriei* Lévl. ssp. *crenata* C. J. Chen. a common Zhuang ethnic group TMP in P. R. China, and compound **14** exhibited weak activities against the K562 (IC_{50}, 12.01 μg/

Table 1. Pharmaceutically Significant PSMs, Precursors, and Main Biosynthetic Pathways

Pharmaceutically significant PSMs	Precursors	Biosynthetic pathways	Subclasses
Alkaloids	Amino acids	Amino acid pathway or mevalonate (MVA) pathway	Pyridine, pyrrole, indole, pyrrolidine, isoquinoline, piperidine alkaloids
Terpenoids	Mevalonic acid or deoxyxyulose	MVA pathway or deoxyxylulose phosphate pathway	Sterols, triterpenes, saponins, essential oils
Phenolics	Phenylpropionic acid	Styrene pathway or shikimate pathway	Flavones, phenols, tannins, coumarins

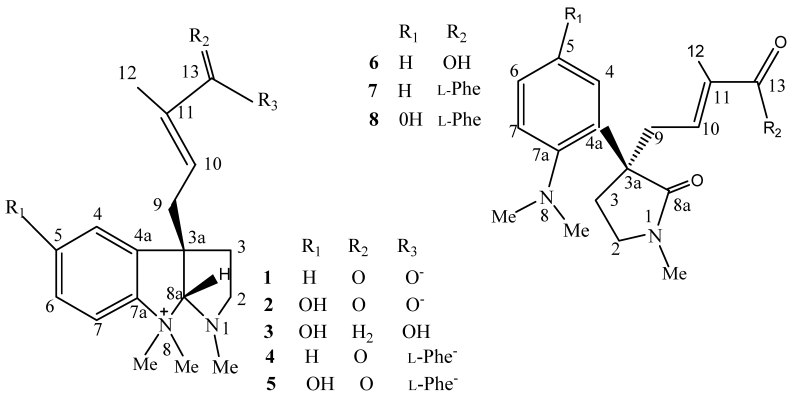

Fig. 1. Eight New Pyrrolidinoindoline Alkaloids (**1–8**)

Table 2. Pharmacological Properties of Alkaloids from TMPs and Subclasses of Active Compounds

Pharmacological property	Compound	Subclass	Use in traditional medicine	Botanical name	Reference
Anticancer	(−)-(2*R**,3*S**,6*S**)-*N*,2-Dimethyl-3-hydroxy-6-(9-phenylnonyl)piperidine	Piperidine alkaloid	Analgesic, antitumor, and pesticide agent	*Arisaema decipiens*	Zhao *et al.*[23]
Antimicrobial	Vanessine	Quinolone alkaloid	Respiratory disorders, urinary disease, stimulant, emetic, diuretic, external wound-cleansing and -healing agent	*Waltheria douradinha*	Gressler *et al.*[24]
Inhibitory cytotoxic	Chabamide (COLO-205 cell line; IC_{50}, 3.10 mg/mL)	Dimeric amide alkaloid	Treatment of diabetes mellitus, hypoglycemic, antidiabetic	*Piper chaba*	Rao *et al.*[25]
	Angustilobine C (KB cells; IC_{50}, 7.76 μg/mL)	Angustilobine alkaloid	Treatment of malaria and dysentery	*Alstonia angustiloba*	Ku *et al.*[26]
	(2 *R**,3 *S**,5 *S**)-*N*,2-Dimethyl-3-hydroxy-5-(10-phenyldecyl)pyrrolidine (K562 cells; IC_{50}, 12.5 μM)	Pyrrolidine alkaloid	Antiinflammatory agent and treatment of snake bite	*Arisaema franchetianum*	Su *et al.*[27]
AChE-inhibitory activity	(+)-1-Nitroapocavidine, berberine, palmatine, dehydrocavidine, sanguinarine	Isoquinoline alkaloid	Treatment of hepatitis, diarrhea, stomachache, and bleeding hemorrhoids	*Corydalis saxicola*	Huang *et al.*[28]
Antioxidative	Caulophylline E	Dihydroazafluoranthene alkaloid	Treatment of external injuries and irregular menses	*Caulophyllum robustum*	Wang *et al.*[29]

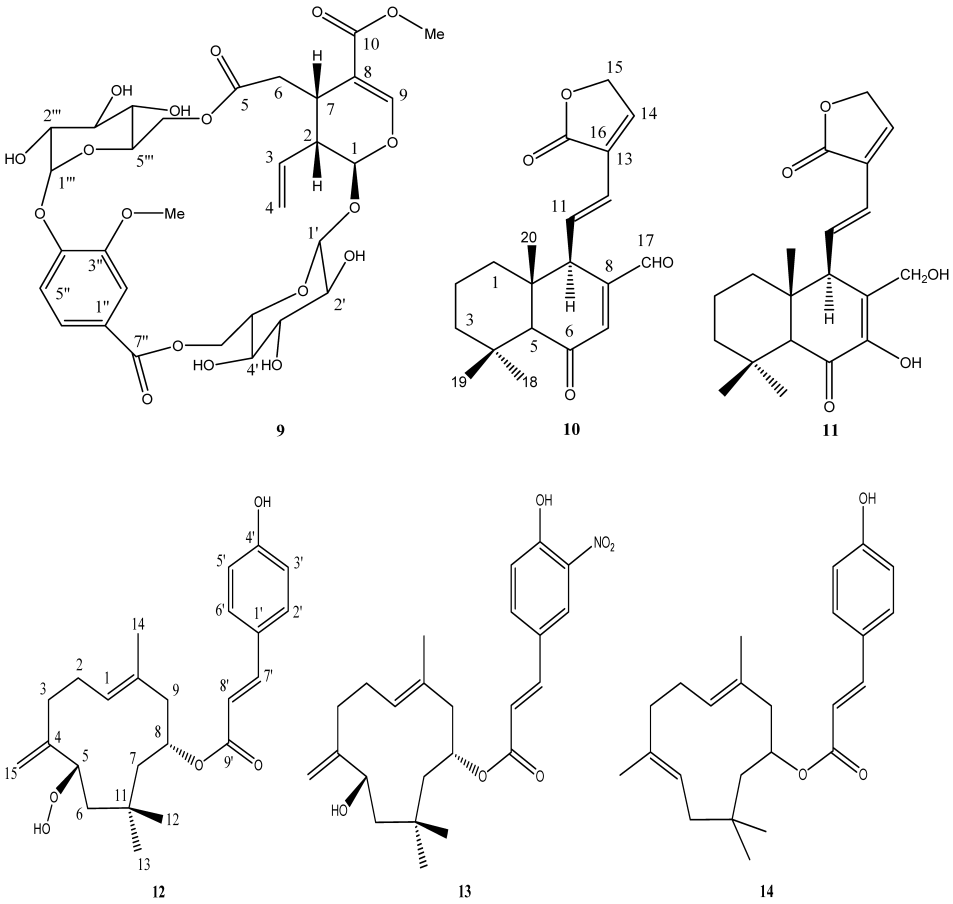

Fig. 2. Structures of Terpenoids Isolated from TMPs

Naresuanoside (**9**) showed a significant inhibitory effects against AchE. Two novel labdane diterpenes (**10**, **11**) showed moderate cytotoxicity against A-549, SK-N-SH, MCF-7, and HeLa cell lines. Compound **14** exhibited weak activities against K562, AGZY, and A549 cell lines.

mL), AGZY (IC_{50}, 27.82 μg/mL), and A549 (IC_{50}, 25.60 μg/mL) cell lines.[33] These results reflect not only the important pharmacological activities of the terpenoids but also reconfirm the important role of TMPs in drug discovery.

2.3. Flavonoids Flavonoids are low molecular-weight compounds. They are extremely important subclasses of phenolic compounds and widely distributed in plants such as vegetables, herbs, spices, and tea. Based on the presence of different substituents on the rings and the degree of benzo-γ-pyrone saturation, flavonoids usually can be separated structurally into flavones, flavanones, and flavonols[2,20,21,34,35] (Fig. 3). Flavonoids display abundant biological effects both *in vivo* and *in vitro*.[36–39] Hence, they are considered as health-promoting substances in the human diet for their antioxidant, antiasthmatic, anticlotting, antiinflammatory, antimicrobial, and anticancer activities.[18]

Many flavonoid compounds have been separated and identified from TMPs, and their remarkable pharmacological properties and multifarious structures have been confirmed. Rao *et al.* isolated two new furanoflavanoids (**15**, **16**) (Fig. 4) from *Derris indica*, a plant used in folk medicine for the treatment of bronchitis, whooping cough, and rheumatic joints.[40] The two compounds displayed moderate intestinal α-glucosidase-inhibitory as well as free radical-scavenging activity.[40] Four flavones, 3,4′-*O*-dimethylquercetin (**17**), 3,7-*O*-dimethylquercetin (**18**), 3-*O*-methylquercetin (**19**), and 3,7,4′-*O*-trimethylquercetin (**20**) (Fig. 4) were isolated from *Siegesbeckia glabrescens*, a plant used in Korean traditional medicine to treat rheumatoid arthritis, asthma, paralysis, and allergic disorders. These compounds are inhibitors of nitric oxide (NO) production in activated microglia (IC_{50} values, 11.1, 4.2, 3.8, 25.1 μM, respectively).[41] From the Thai traditional medicinal plants *Kaempferia parviflora* and *Boesenbergia pandurata*, used for treatment of several inflammatory-related diseases (gout, allergy, apthous ulcer, and peptic ulcer), three compounds including 5-hydroxy-3,7,3′,4′-tetramethoxyflavone (**21**, *K. parviflora*), panduratin A (**22**, *B. pandurata*), and hydroxypanduratin A (**23**, *B. pandurata*) (Fig. 4) exhibited potent activities against the NO-inhibitory effect, *i.e.*, these compounds are potential antiinflammatory agents.[42] In addition, 4′-*O*-methoxy-luteolin-7-*O*-rhamnoglucoside (**24**) (Fig. 4) isolated from *Euphorbia cuneata*, a folk medicine used for the treatment of gastric disorders, demonstrated antiulcerogenic action.[43] These results confirm the potential value of flavonoids from TMPs, both in

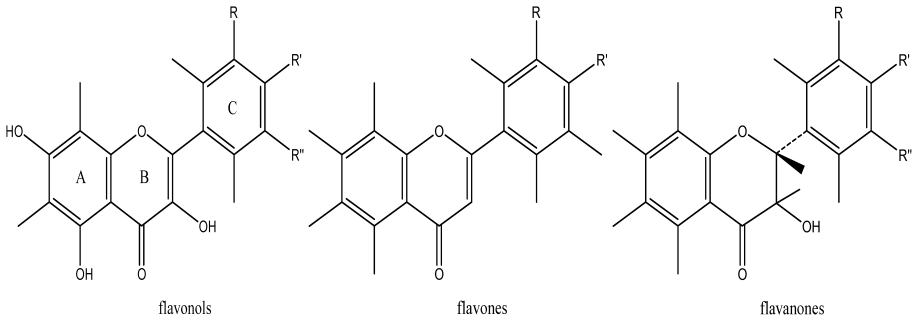

Fig. 3. Structures of Flavonols, Flavones, and Flavanones

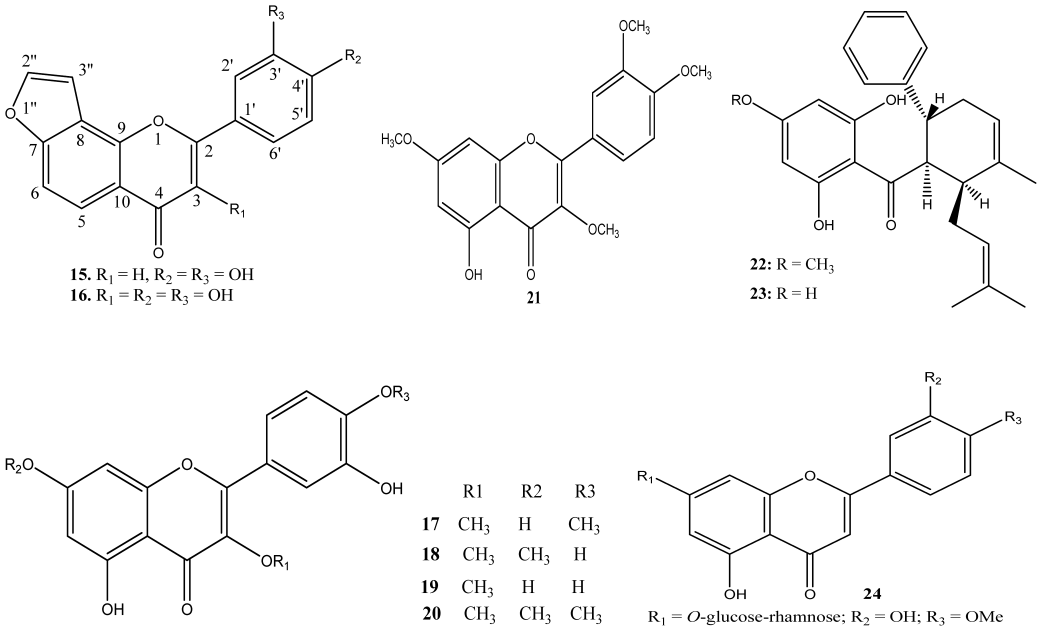

Fig. 4. Structures of Bioactive Flavonoids from TMPs

Two new furanoflavanoids (**15**, **16**) displayed moderate intestinal α-glucosidase-inhibitory and free radical-scavenging activity. Four 3-*O*-methyl-flavones (**17**, **18**, **19**, **20**) were inhibitors of NO production. Compounds **21**, **22**, and **23** all exhibited potent activity against the NO-inhibitory effect. **24** demonstrated antiulcerogenic action.

scientific research and industry.

3. MAJOR PHARMACOLOGICAL ACTIVITIES

As mentioned above, the active constituents from TMPs have potent pharmacologic properties such as antimicrobial, antiinflammatory, analgesic, antioxidative, antiviral, anticancer, antihypertensive, antidiabetic, antiatherosclerotic, antiulcer, hemostatic, cardiotonic, immunomodulatory, and neuroprotective activities.[20,38,44–46] The most important basic effects are antiinfectious, antioxidant, and antiinflammatory.

3.1. Antiinfectious Activities Antiviral, antimalarial, antibacterial, antifungal, and antiparasitic effects are included among antiinfectious activities in this review. TMPs are rich in a wide variety of secondary metabolites that have antiinfectious properties.[21] In 1996, mangostin (**25**) and γ-mangostin (**26**) (Fig. 5) were isolated from *Garcinia mangostana* and showed noncompetitive inhibition of human immunodeficiency virus (HIV)-1 (IC_{50}, 5.12, 4.81 mM, respectively).[47] Subsequently, numerous new pharmacologically active constituents from TMPs with antiinfectious activity have been reported. He *et al.* revealed that glycyrrhizol A and 6,8-diisoprenyl-5,7,4′-trihydroxyisoflavone from *Glycyrrhiza uralensis* exhibited potent antibacterial activity against *Streptococcus mutans* (minimum inhibitory concentraction 1, 2 μg/mL, respectively).[48] Digiferruginol and hederagenin from *Chirita longgangensis* var. *hongyao* showed weak effects against porcine respiratory and reproductive syndrome virus (PRRSV).[48] This result could provide a pharmacological basis to inhibit PRRSV replication.[27] In addition, many other plant-derived substances from TMPs such as *Murraya koenigii*, *Erythrina caffra*, *Corydalis saxicola*, *Larrea tridentate*, *Holarrhena antidysenterica*, and *Melampyrum arvense* exerted antiviral,[49,50] antibacterial,[51,52] and antiprotozoal[53,54] activities.

3.2. Antioxidant Activities Numerous physiological processes can produce oxygen-centered free radicals in the human body. With the accumulation of reactive oxygen species, some free radicals can cause oxidative damage to biomolecules (*e.g.*, lipids, proteins, DNA), triggering cancer, diabetes, atherosclerosis, aging, and other degenerative disorders.[20,55,56] Hence, antioxidant activity is considered a fundamental property for human health and has attracted widespread research interest. Many plant-derived substances, such as alkaloids, terpeniods, and flavonoids that exhibit potent antioxidant effects can scavenge free radicals.

Based on the 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic) acid radical and 1,1-diphenyl-2-picrylhydrazyl radical (DPPH), many pharmacological constituents with antioxidant activity have been identified from TMPs. Luteolin from the flower of *Chrysanthemum sinense* showed significant xanthine oxidase activity (IC_{50}, 1.3 mM), which is stronger than that of the clinically used drug allopurinol (IC_{50}, 2.5 mM).[57] Two new furanoflavanoids (**15**, **16**) (Fig. 4) from *D. indica*, a plant used in folk medicine for the treatment of bronchitis, whooping cough, and rheumatic joints, displayed potent DPPH radical-scavenging activity.[40] Additionally, the new dihydroazafluoranthene alkaloid caulophylline E (**27**) (Fig. 5) was isolated from the roots of *C. robustum*, which is used as a traditional Chinese medicine to treat external injuries and irregular menses, and showed good scavenging effects against DPPH radicals with an IC_{50} value of 39 μM.[58] Furthermore, numerous extracts from TMPs showed marked antioxidant activity, such as *Kleinhovia hospita*,[59] *Orthosiphon stamineus*,[60] *Anogeissus acuminata*,[61] *etc.*

3.3. Antiinflammatory Effects Many different stimulating factors can induce inflammation, like physical damage, microbial invasion, toxic substances, and immune responses.[45,62] Generally, nonsteroidal antiinflammatory drugs and corticosteroids are prescribed for treating inflammatory disorders worldwide. However, after the discovery of two mediators of inflammation, prostaglandins and NO, more attention has focused on TMPs,[63] and numerous bioactive ingredients in TMPs have been evaluated for possible antiinflammatory activity.

Tewtrakul *et al.*[64] isolated four NO-inhibitory ingredients from *Derris trifoliata* stems and found that 12α-hydroxyrotenone (**28**) had very potent NO-inhibitory activity with an IC_{50} value of 0.002 μM, followed by deguelin (**29**, 0.008 μM), 12α-hydroxyelliptone (**30**, 0.010 μM), and α-toxicarol (**31**, 0.013 μM) (Fig. 5). Diosgenin-3-*O*-α-L-rhamnosyl (1→2)-β-D-glucopyranoside (**32**), dioscoreanone (**33**), and dioscorealide B (**34**) were isolated from *Dioscorea membranacea* and showed potent NO-inhibitory activity (IC_{50} value, 3.5, 9.8, 24.9 μM, respectively) (Fig. 5).[65] Two xanthones, α- and γ-mangostins (**35** and **36**) (Fig. 5), were isolated from the fruit hull of *G. mangostana*, which is used as a folk antiinflammatory agent in Southeast Asia, and both significantly inhibited NO production (IC_{50} value, 12.4, 10.1 μM, respectively).[66] Moreover, aeschynanthoside D (**37**) and naringenin (**38**) (Fig. 5) isolated from *Aeschynanthus bracteatus* showed weak dose-dependent inhibitory activities against lipopolysaccharide-induced NO production.[66] These results demonstrate that pharmacologically active ingredients from TMPs may play important and indispensable roles as lead pharmaceuticals for preventing and treating inflammatory diseases.

4. STRUCTURE–ACTIVITY RELATIONSHIPS (SAR)

The preceding sections summarized the importance of TMPs, both as pharmaceutical agents and/or as leads to biology-oriented synthesis. However, the specific pharmacological properties of compounds are governed by their chemical structures including scaffolds and number, positions, and types of substitutions, that is, structure determines activity. Therefore, the study of SARs is a necessity when attempting to discover pharmacologically active constituents and undertaking biology-oriented synthesis. In recent years, a number of SAR studies have been undertaken, most of which focused on flavonoids and alkaloids.

4.1. SAR Studies on Pharmacological Activity of Flavonoids Flavonoids and Antioxidant Activity: Flavonoids have widely been considered to be a class of efficient antioxidants. In 1998, Brown *et al.*[67] proposed the SAR of flavonoid antioxidant activity by comparing quercetin, rutin, kaempferol, and luteolin. Teixeira *et al.*[68] found that a catechol group in ring B and a 3-hydroxy group in ring C were essential for the antioxidant activity (Fig. 6). In general, the number and position of hydrogen-donating hydroxyl groups on the aromatic ring of the flavonoid molecules determine their free radical-scavenging and antioxidant activity, although other factors can be involved, such as glycosylation of aglycones or

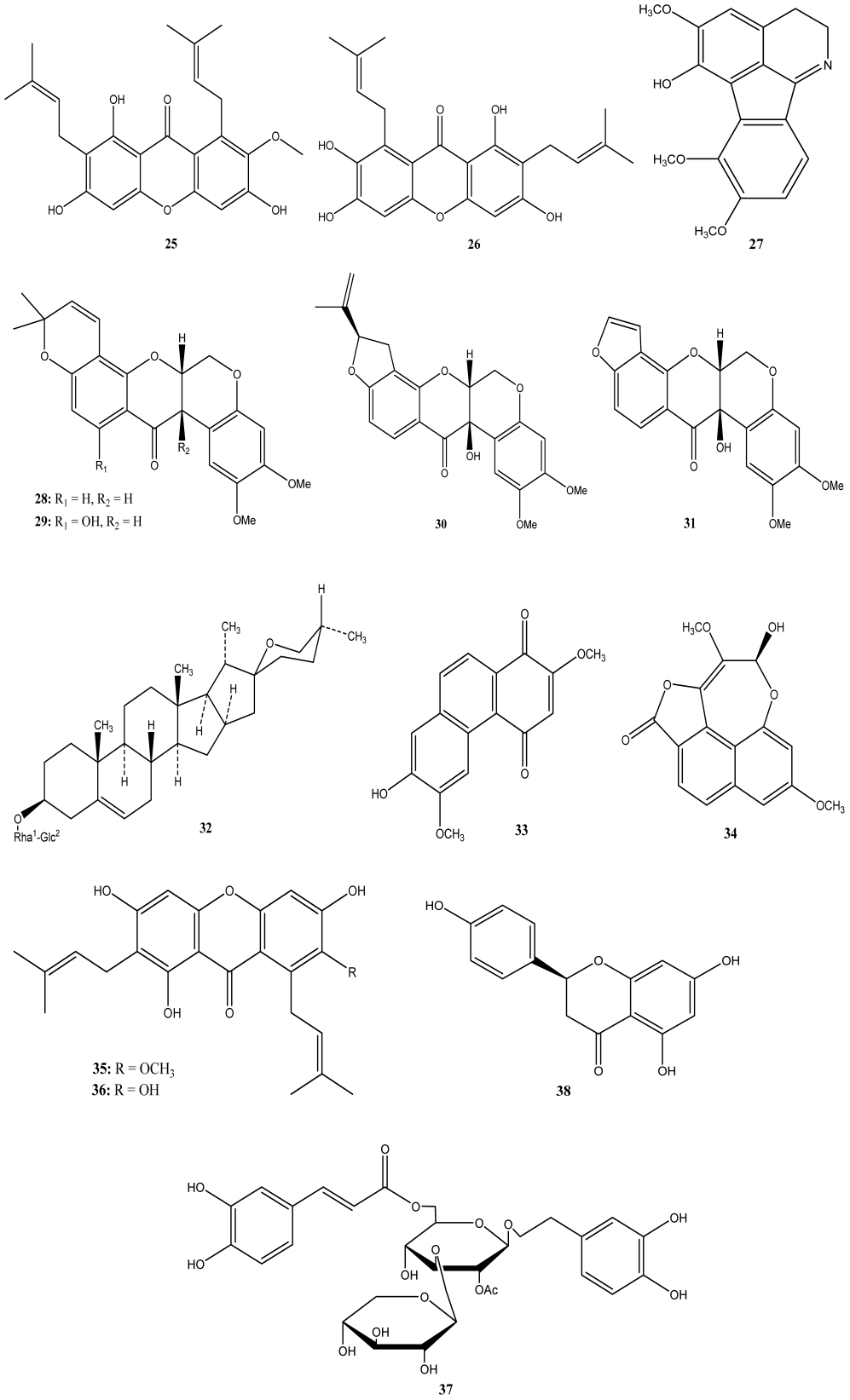

Fig. 5. Structures of Some Active Constituents of TMPs Showed Antiinfectious, Antioxidant, and Antiinflammatory Activity

Compounds **25** and **26** showed inhibition against HIV-1; **27** showed good DPPH-scavenging effects; and the other compounds all showed NO-inhibitory activity.

other H-donating groups (–SH, –NH). For example, flavonoids with multiple hydroxyl group structures have high antioxidant activity (*e.g.*, quercetin, myricetin, and kaemperol), and the glycosylation of flavonoids reduces their activity (*e.g.*, rutin, myricitrin, and astragalin).[20,69] Recently, two rotenoids, 6a,12a-dehydrodeguelin (**39**) and deguelin (**40**) (Fig. 6), were isolated from *D. trifoliata* stems. Both showed potent activity against DPPH with an IC_{50} value of 7.4 and 27.4 μM, respec-

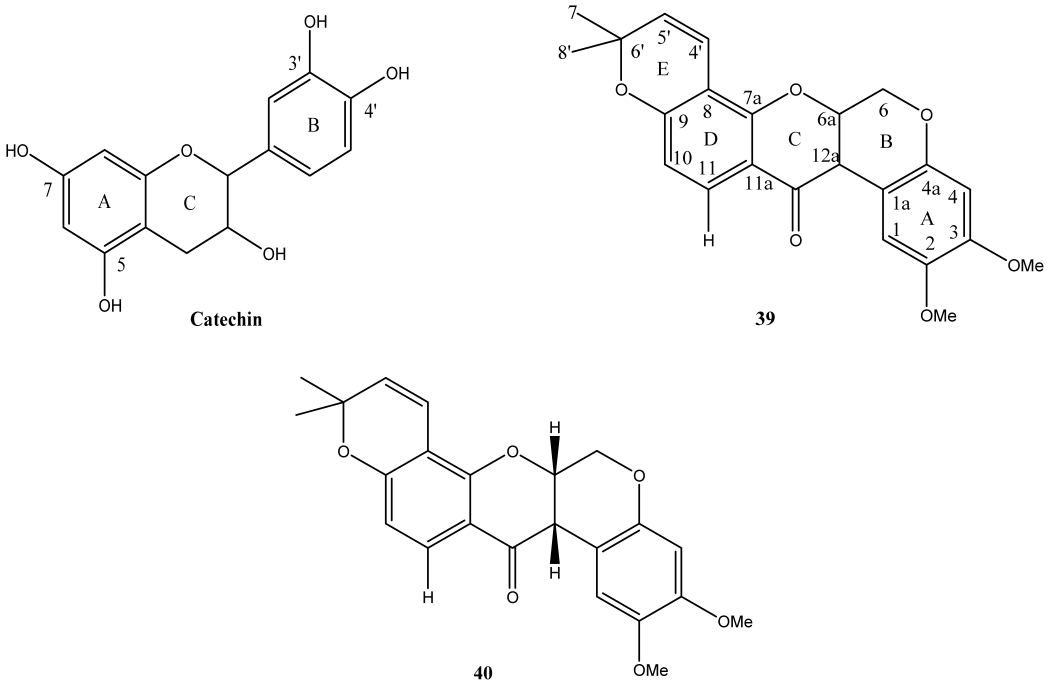

Fig. 6. SARs of Flavonoids Determine Antioxidant Activity

Studies showed that a catechol group in ring B and a 3-hydroxy group in ring C were essential for the antioxidant activity, as occurs in catechin. Two rotenoids (**39**, **40**) with a double bond at C6a–C12a showed increased DPPH radical-scavenging activity, and hydroxylation of C11 at the D-ring decreased it.

tively. When the SAR of the two rotenoids was examined, it was found that the introduction of a double bond at C6a–C12a increased their DPPH radical-scavenging activity and hydroxylation of C11 at the D-ring decreased it.[64)]

Flavonoids and Antiinflammatory Activity: Flavonoids exhibit antiinflammatory activity, and the SAR of this property has been elaborated. Nine flavonoids with different structural features were selected to characterize their effects on the expression of cyclooxygenase 2 (COX2) and inducible nitric oxide synthase (iNOS). Both targets are correlated with inflammatory processes.[70)] The results indicated that: 1) the position of the B ring in 2- and 4′-hydroxylation favors inhibition in both cases; 2) the presence of 3-OH is detrimental to iNOS inhibition (kaempferol *vs.* apigenin) but may be compensated for by additional B ring hydroxylation (quercetin *vs.* luteolin); 3) flavonols tend to increase COX2 expression, and, with the exception of quercetin, overall hydroxylation may counteract the influence of 3-OH; and 4) the effect of 3′-hydroxylation appears to be detrimental.[70)] Moreover, when the SARs of the four rotenoids (**28**–**31**) with NO-inhibitory activity isolated from *D. trifoliata* stems were examined, it was found that: 1) hydroxylation at C12a dramatically increased NO release; 2) prenylation at the furan ring increased NO release markedly; and 3) hydrogenation of the double bond at C6a–C12a conferred higher NO release activity.[64)]

Furthermore, some SAR studies on the antiproliferative activity of flavonoids indicated that the presence of a C-2,3-double bond and the number and position of hydroxylations of the B-ring were essential for their antiproliferative activities.[70,71)]

4.2. SAR Studies on the Pharmacological Activity of Alkaloids Alkaloids are significant pharmacologically active constituents. SAR studies on alkaloids can provide templates for the synthesis or semisynthesis of new drugs. However, the structures of alkaloids are more complex than those of flavonoids, and their diversity produces many challenges when performing SAR studies. Although difficulties exist, several related studies have been carried out.

Four carbazole alkaloids, ekeberginine (**41**), methyl carbazole-3-carboxylate (**42**), clausine E (**43**), and *O*-demethylmurrayanine (**44**), were isolated from *Clausena anisata* (Fig. 7). All compounds were tested for antitumor-promoting activity in a short-term *in vitro* assay of the tumor promoter 12-*O*-tetradecanoylphorbol-13-acetate-induced Epstein–Barr virus early antigen (EBV-EA) activation in Raji cells. Among these compounds, **41**, **42**, and **43** lacking the prenyl side chain showed lower EBV-EA-inhibitory activities than **40** with a prenyl group at C-4. SAR analysis of antitumor carbazole alkaloids showed that the prenyl group at C-4 plays an important role in the inhibitory activity.[72)] Moreover, a new nitro-containing tetrahydroprotoberberine, (−)-2,9-dihydroxyl-3,11-dimethoxy-1,10-dinitrotetrahydroprotoberberine (**45**), and five isoquinoline alkaloids, (+)-1-nitroapocavidine (**46**), berberine (**47**), palmatine (**48**), dehydrocavidine (**49**), and sanguinarine (**50**) (Fig. 8), were isolated from the whole plant of *C. saxicola*. All compounds showed potent inhibitory activity against AchE with IC_{50} values of less than $10\,\mu$M. Further SAR studies demonstrated that the increase in anti-AchE activity was attributed to the nitro-substituent at ring A in the tetrahydroprotoberberines.[72)] In addition, three berberine alkaloids from *Coptis chinensis*, berberine (**47**), coptisine (**51**), and palmatine (**48**) (Fig. 7), were evaluated for their inhibitory activity against *Escherichia coli*. The results showed that the sequence of their antimicrobial activity was berberine>coptisine>palmatine. The SAR study showed that the antimicrobial activity depended on the substituent group on phenyl rings C2 and C3, and methylenedioxy improved antimicrobial activity more markedly than methoxyl.[73)]

4.3. SAR Studies on the Pharmacological Activity of

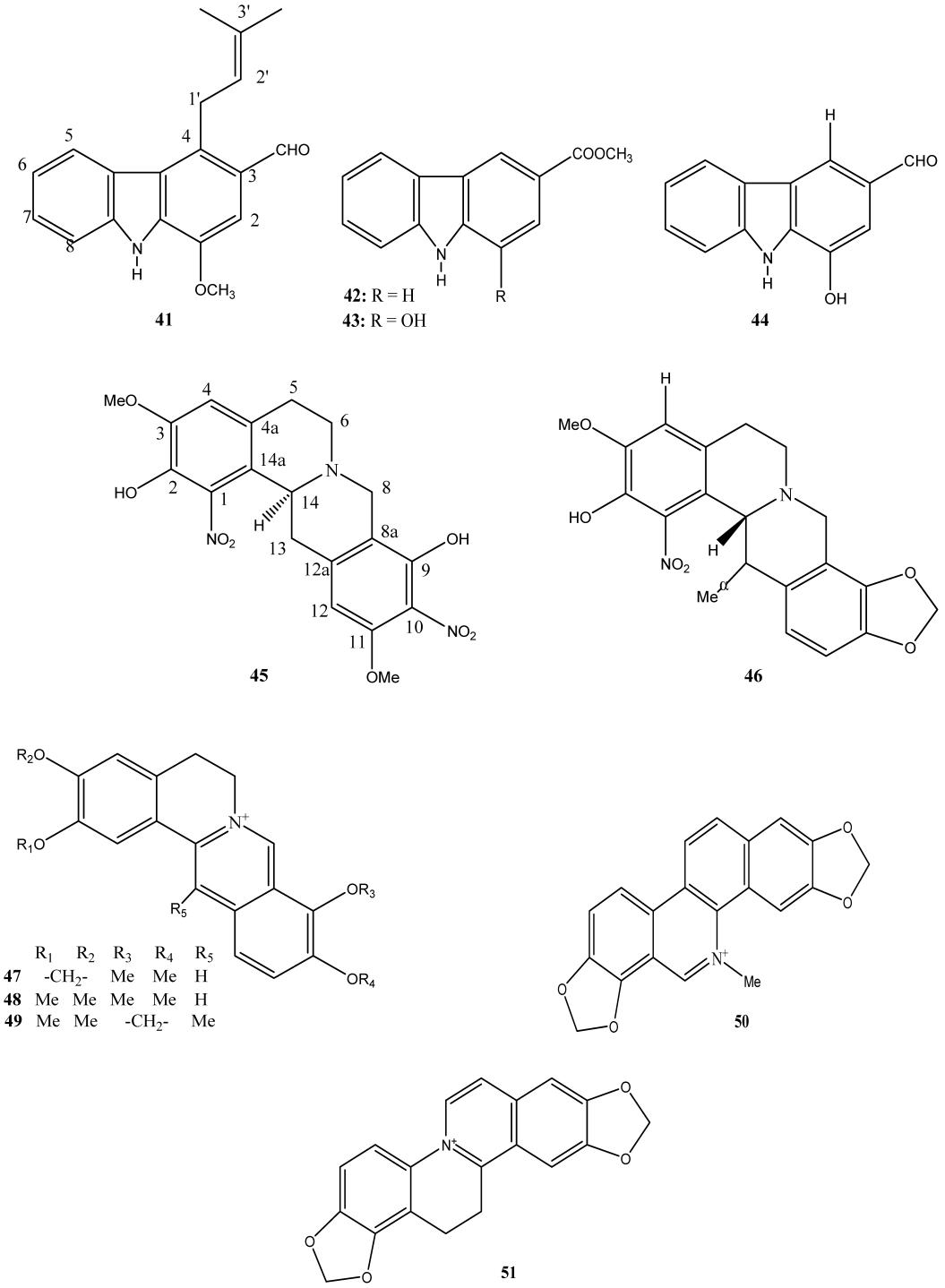

Fig. 7. Results of SAR Studies in Alkaloids

SAR studies on antitumor carbazole alkaloids (**41**–**44**) showed that a prenyl group at C-4 plays an important role in the inhibitory activity. SAR studies on a new nitro-containing tetrahydroprotoberberine (**45**) and five isoquinoline alkaloids (**46**–**50**) showed that nitro substituents at ring A improve anti-AchE activity. Comparing the antimicrobial activity of **48**, **49**, and **51**, the SAR results showed that the substituent group on phenyl rings C2 and C3 as well as methylenedioxy improves antimicrobial activity more markedly than methoxyl.

Other Bioactive Constituents In addition to those mentioned above, other constituents have also been subjected to SAR studies. Reddy *et al.*[74] used hedychenone (**52**) (Fig. 8), a plant-derived labdane diterpenoid with potent *in vitro* cytotoxic activity against cancer cells, as a template to synthesize a series of analogues. The majority of the analogues displayed more potent activity than the parent compound, and SAR studies indicated that a furanoid ring has a greater impact on cytotoxicity than the decalone nucleus. Dimerization through C-8 significantly enhanced the cytotoxic activity of the hedychenone.[74] SAR studies on the active principles in the NO-inhibitory activity of *D. membranacea* revealed that diosgenin-3-*O*-*α*-L-rhamnosyl (1→2)-*β*-D-glucopyranoside (**31**) (Fig. 5) with a rhamnoglucosyl moiety at C-3 exhibited much higher activity.[65]

It is not surprising that SAR studies provide information

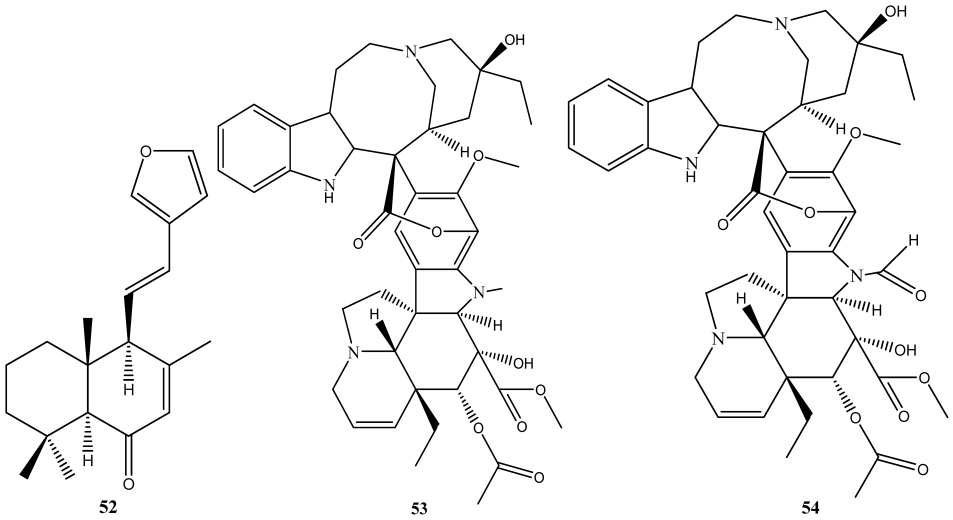

Fig. 8. Examples of Plant-Derived Compounds with Cytotoxic Activity: Hedychenone (**52**), Vinblastine (**53**), and Vincristine (**54**)

for further lead optimization of some classes of compounds as new pharmaceuticals. Hence, SAR studies of bioactive constituents are essential for the discovery of new pharmcologically active ingredients.

5. GENERAL APPROACHES TO ACTIVE INGREDIENT DISCOVERY

Pharmacologically active constituents from TMPs continued to provide an important source of novel drug leads, and methods for obtaining plant-derived substances are an important research topic because many downstream studies (like pharmacology, SAR, synthesis, *etc.*) are based on a single ingredient. Generally, these approaches can be summarized as: random selection followed by chemical screening; random selection followed by one or more biologic assays; follow-up of biologic activity reports; and follow-up of ethnomedical (traditional medicinal) uses of plants.[5] All of the approaches involve multifaceted methods combining botanical, phytochemical, biological, and molecular techniques. The approach of random selection followed by chemical screening, also called bioassay-guided fractionation, has been used in the past and in developing countries continues to be the main method followed. However, based on the literature and current practice, the traditional bioassay-guided fractionation is evolving into another approach, tentatively called "random-bioassay fractionation." Hence, the two most common basic approaches, bioassay-guided fractionation and random-bioassay fractionation, are utilized extensively at present (Fig. 9).

Although numerous methods can be used to discover active compounds from TMPs, phytochemists and pharmacologists encounter numerous challenges, one of which is the procurement of plant materials with a history of folk or indigenous medicinal practice. The following section describes a rational strategy to overcome those challenges.

6. MODERN ETHNOBOTANY AND PHARMACOLOGICALLY ACTIVE CONSTITUENT DISCOVERY

The word "ethnobotany" first emerged in print in 1896 and was defined as research on "plants used by primitive and aboriginal people" by William Harshberger, the well-known U.S. botanist.[75] Currently, it has been expanded to a more comprehensive definition: "The study of the relationships between man and his ambient vegetation."[6] "Modern ethnobotany" is a stage of ethnobotanical development which emphasizes the study of interrelationships between local populations and plants using modern technologies.[12] It can provide more scientific evidence in support of traditional plant usage and enrich the theory and methodology. TMPs, one of the main research interests of ethnobotany, have made a significant contribution to the theoretical development of the field after ethnobotanical investigation.

PSMs have been the source of many active ingredients in medicines. The discovery of these pharmacologically active constituents was usually based on ethnobotanical information, and many drugs used today were developed from medicinal plants employed in indigenous societies,[75] *e.g.*, Gyllenhaal *et al.* compared the ethnobotanical and random approaches in the search for new bioactive compounds.[77] The results indicated that ethnomedicinal uses may contribute to a higher rate of activity in drug discovery screening. There were several other reasons supporting the discovery of pharmacologically active ingredients from TMPs *via* modern ethnobotany:

1) Ethnobotanical inquiry can provide abundant materials for laboratory experiments. Ethnobotanical interviews are a shortcut for chemists to select which plants should be collected for phytochemical research and for pharmacologists to screen plants with pharmacologically active compounds.
2) Ethnobotanical study is a significant source of novel active constituents and remedies. While documented knowledge of traditional medicine is essential to human health, many important undocumented folk or indigenous practices may be rare and could easily disappear. Because local knowledge is not based on scientific research, it may involve novel active constituents and remedies. Nevertheless, ethnobotanical studies generally result in the description of a rather limited set of well-documented useful plants, particularly medicinal plants.[75]
3) Ethnobotanical research can integrate cultural diversity in folk therapies. In the northern hemisphere, ethnic groups

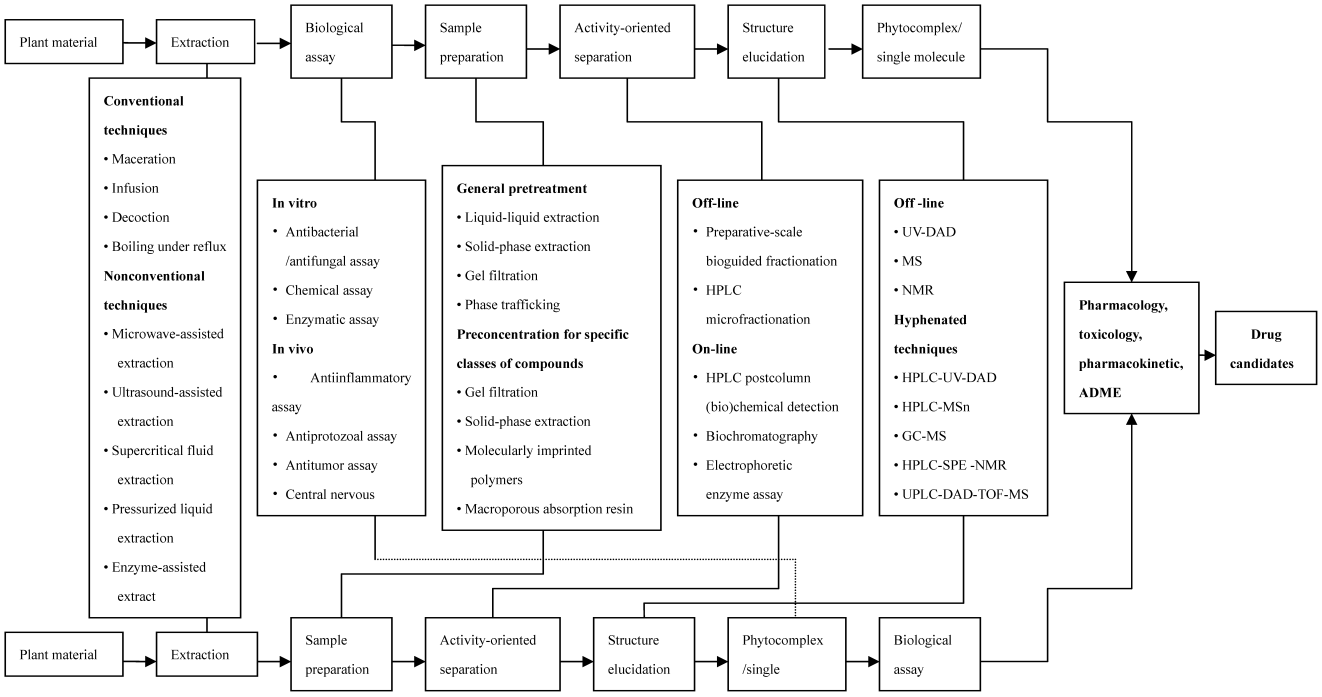

Fig. 9. Two Most Common Basic Approaches to Active Constituent Discovery

Adapted from Brusotti *et al.*[76]

became widely separated after a long history of migrations but nevertheless remained culturally and genetically related. They also depended on strikingly similar medicinal flora.[5] This indicates that the selection of medicinal plants and direct experience with their bioactivity are affected by cultural differences. In addition, medical publications that convey a variety of cultural values rather than biochemistry results also affect the medicinal plant options.[78] Hence, semistructured interviews and individual discussions provide opportunities to integrate the cultural diversity of medicinal plant usage.

Based on the reasons above, ethnobotany is an efficient, rational strategy for the discovery of pharmacologically active ingredients from TMPs. Numerous successful cases have demonstrated the utility of this strategy. The best classical example is that of *Catharanthus roseus* (Apocynaceae), used by people indigenous to southeastern Madagascar as an oral hypoglycemic agent. After laboratory research was conducted on the plant, it was found to contain more than 75 alkaloids, among which vinblastine (**52**) and vincristine (**53**) (Fig. 8) have shown high rates of success in treating childhood leukemia and Hodgkin's disease.

In recent years, our group has performed many studies to discover pharmacologically active ingredients from TMPs *via* ethnobotanical approaches. In Jingxi county, southwestern Guangxi region, southern China, for example, recorded interviews were conducted on more than 400 medicinal plant species. In addition, based on the indigenous knowledge of different medicinal cultures in Jingxi from the Zhuang, Han, Miao, and Yao ethnic groups,[79] many TMPs (such as *S. moellendorfii*, *C. saxicola*, *Striga asiatica*, *etc.*) have been studied in the laboratory. A massive group of novel pharmacologically active compounds were isolated and tested. Moreover, analogous research has been increasing elsewhere, especially in developing countries like India, Laos,[80] Malaysia,[81] Thailand,[82] Indonesia, and many countries in Africa and South America.

Thus it can be seen that the investigation of TMPs using the ethnobotanical approach can lead to new biodynamic compounds that may have significant applications in society.

7. CONCLUSION

This paper gives an overview of pharmacologically active constituents from TMPs, including recent research on their metabolic pathways, pharmacological properties, SARs, and approaches. Strategies for the discovery of pharmacologically active ingredients from TMPs using ethnobotanical approaches are discussed. Indigenous people understand that TMPs can be effective for the treatment of specific diseases based on experience passed on verbally over generations. Ethnobotanical approaches can be shortcuts to search such TMPs for scientific studies, like phytochemistry, pharmacology, *etc.*

The potential lack of reproducibility of results, however, is a pitfall that should be taken into account when exploring pharmacologically active constituents from TMPs. As individual organisms, plants have inherent growth cycles and variability in chemistry at certain growth stages which may result in poor reproducibility of bioactivity. Plants harvested in different environments and at different times can also vary in their bioactive secondary metabolites, both qualitatively and quantitatively. Hence, it may be difficult to obtain the same pharmacologically active constituents from the same plants unless they are collected in the same growth stage and environment. It appears more rational to seek pharmacologically active ingredients from TMPs which have the same properties and structures but not necessarily the same content. The

content of pharmacologically active ingredients can be improved or acquired by other approaches, like plant cell culture, chemical synthesis or semisynthesis, and molecular biological techniques or metagenomic approaches applied to bacteria.[1,83)]

In summary, pharmacologically active constituents from TMPs have widely varying structures and pharmacological activity. Numerous medicinal agents and templates for synthesis have been discovered from TMPs. Even with the challenges facing active constituent discovery from TMPs, they will continue contributing to the development of treatments. Moreover, with folk knowledge of medicine vanishing and interest in indigenous practices increasing, strategies for the discovery of pharmacologically active ingredients from TMPs through ethnobotanical surveys must be adopted.

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