Progress in Chemical Constituents and Bioactivities of *Broussonetia papyrifera* (L.) Vent

Fang Li,^a Tingyu Wen,^a and Jifeng Liu^{*,a,b}

^a School of Pharmaceutical Sciences; Institute of Drug Discovery and Development; Key Laboratory of Advanced Pharmaceutical Technology, Ministry of Education of China; Zhengzhou University, Zhengzhou, Henan 450001, China

^b Collaborative Innovation Center of New Drug Research and Safety Evaluation, Zhengzhou, Henan 450001, China

Email: liujf2009y@126.com (J. L.)

Abstract *Broussonetia papyrifera* (*B. papyrifera*) is a deciduous tree of the genus *Broussonetia* of Moraceae. Its roots, stems, leaves, skins, fruits and seeds can be used as folk medicine. Modern technology research showed that the *B. papyrifera* was rich in flavonoids and triterpenoids, and its components exhibited anti-proliferation, antibacterial, anti-oxidation, cytotoxicity, enzyme inhibition and other activities. In this paper, we summarized the progress in the chemical constituents and bioactivities of *B. papyrifera* since 2000 to provide ideas for the medicinal development prospects of *B. papyrifera*.

Keywords *Broussonetia papyrifera*, chemical constituents, bioactivities, progress

Introduction

Broussonetia papyrifera (L.) Vent, belonging to the deciduous tree of the genus *Broussonetia* of Moraceae, is also known as paper mulberry. There are five species in the world, which distributed mainly in eastern Asia and the Pacific islands. Four species distributed in the Yellow River, the Yangtze River, the Pearl River and Taiwan in China, including *B. papyrifera*, *B. kazinoki*, *B. kaempferi*, and *B. kurzii*. *B. papyrifera* is a highly valued plant in China. Its leaves, roots, barks and fruits are traditional Chinese medicines with multiple bioactivities. The roots can be used as diuretics, tonics and inhibitors;^[1] the branches can be used to treat rubella, red eyes and swelling, and urinary dysfunction; the leaves have the effects of clearing away heat and detoxifying, cooling blood and diuresis,^[2] and can be used to treat blood vomiting, dysentery, hemorrhoids, *et al.*; the fruits are also known as *Fructus Broussonetiae*, which have the functions of tonifying kidney, clearing liver, eyesight and diuretic,^[3,4] and can be used to treat fatigue, severe fever, and dizziness.^[5] Modern pharmacological studies revealed that *B. papyrifera* had the activities of anti-inflammatory,^[6] anti-tumor,^[7] anti-fungal,^[8] anti-hepatic toxicity,^[9,10] anti-oxidation,^[11] enhancing immune function,^[12] improving endoplasmic reticulum stress in the Alzheimer's disease model.^[13]

Herein, we utilized "chemical constituents of *Broussonetia papyrifera*" and "activities of *Broussonetia papyrifera*" as keywords to search for relevant literatures and books in SciFinder, Web of Science, China National Knowledge Infrastructure academic database and website, and has summarized chemical constituents and bioactivities of *B. papyrifera* since 2000.

Chemical Constituents

Since 2000, researchers have isolated **72** compounds from various parts of the *B. papyrifera*, including flavonoids, terpenoids, phenylpropanoids, anthraquinones and organic acids.

Flavonoids and their glycosides in *B. papyrifera* distributed widely in barks, which were identified as kazinol B (**1**), 7,4'-

dihydroxy-3'-prenylflavan (**2**), 7,3'-dihydroxy-4'-methoxyflavan-**(3)**, kazinol A (**4**), 3'-(3-methylbut-2-enyl)-3',4',7-trihydroxyflavane (**5**), brousochalcone A (**6**), 5,7,3',4'-tetrahydroxy-3-methoxy-8-geranylflavone (**7**), 5,7,3',4'-tetrahydroxy-3-methoxy-8,5'-diprenylflavone (**8**), uralenol (**9**), papyriflavonol A (**10**) and brousoflavonol B (**11**). Above-mentioned compound **3** was isolated from the barks for the first time and compounds **7** and **8** were two new prenylflavones.^[14-16] Besides, brousochalcone B (**12**), 3,4-dihydroxisolonchocarpin (**13**), 4-hydroxisolonchocarpin (**14**), kazinol E (**15**), 8-(1,1-dimethylallyl)-5'-(3-methylbut-2-enyl)-3',4',5,7-tetrahydroxyflavonol (**16**), brousoflurenone A (**17**) and brousoflurenone B (**18**) were isolated from roots, in which compounds **17** and **18** were two novel benzofluorenes.^[17]

Besides the above-mentioned compounds, flavonoids and their glycosides were also isolated from the fruits and leaves of *B. papyrifera*, which were named apigenin (**19**), apigenin-7-O- β -D-glucopyranoside (**20**), broupapyrin A (**21**), cosmosiin (**22**), luteolin-7-O- β -D-glucopyranoside (**23**), and apigenin-6-C- β -D-glucopyranoside (**24**). Compound **21** was a new isoprenylated flavanol^[18] and **24** was isolated from the leaves for the first time.^[19,20] Their structures are shown in Figure 1.

Triterpenoids and their glycosides

Since 2000, nine triterpenoids have been isolated from *B. papyrifera*, named lupeol acetate (**25**), (3 β)-3-(acetyloxy)-eupha-7,25-dien-24-one (**26**), (3 β ,24R)-3-(acetyloxy)-eupha-7,25-dien-24-ol (**27**), (3 β ,24S)-eupha-7,25-diene-3,24-diol (**28**), (3 β ,24R)-eupha-7,25-diene-3,24-diol (**29**), 3 β -acetoxy-

Figure 1 Chemical structures of flavonoids isolated from *B. papyrifera*.

tirucalla-7-en-24S,25-diol (**30**), oleanolic acid (**31**), augustic acid (**32**). Among them, compounds **26**–**30** were five new tetracyclic triterpenoids.^[14,21–23] The structures are shown in Figure 2.

Figure 2 Chemical structures of triterpenoids isolated from *B. papyrifera*.

Phenylpropanoids

Eight coumarins and four lignans have been isolated from *B. papyrifera*, named 6,7-dimethoxycoumarin (**33**), (+)-marmesin (**34**), (+)-(2'S,3'R)-3-hydroxyl marmesin (**35**), (+)-pinoresinol-4'-O-β-D-glucopyranosyl-4"-O-β-D-apiofuranoside (**36**), lirioidendrin (**37**), lariciresinol-9-O-β-D-glucopyranoside (**38**), syringaresinol-4-O-β-D-glucopyranoside (**39**), 3,4',5'-trihydroxy-5-methoxy-6H-benzo [c] chromen-6-one (**40**), alternariol 4'-O-methyl ether (**41**), alternariol 5-O-methyl ether (**42**), alternariol (**43**), alternuene (**44**). Compounds **34** and **35** were isolated from the barks for the first time, compound **36** was a new lignan, compounds **38** and **39** were lignans isolated from the leaves for the first time.^[20,21,24,25] Their structures are shown in Figure 3.

Organic acids and their esters

Nine organic acids and their esters have been isolated from *B. papyrifera*, which were identified as daucosterol palmitate (**45**), palmitic acid ethyl ester (**46**), palmitic acid (**47**), linoleic acid (**48**), 9-octadecenoic acid (**49**), 8,11-octadecadienoic acid

Figure 3 Chemical structures of phenylpropanoids isolated from *B. papyrifera*.

Figure 4 Chemical structures of organic acids and their esters isolated from *B. papyrifera*.

(50), α -monopalmitin (51), monoheptadecanoin (52) and heptadecanoic acid (53). Among them, compounds 46, 47 and 48 were isolated from the fruits for the first time, compounds 51 and 52 were isolated from the barks firstly, and compound 53 was isolated from the leaves for the first time.^[19,21,25,26] Their structures are shown in Figure 4.

Other compounds

Some other types of compounds were also isolated from *B. papyrifera*, including anthraquinones, alkaloids, sterols, aryl propanes, lactones, which were identified as physcion (54), β -daucosterol (55), phytol (56),^[19] altertoxin IV (57), altersolanol A (58), altersolanol C (59), altertoxin I (60), ergosta-4,6,8,22-tetraen-3-one (61),^[24] β -sitosterol (62), broussonin A (63), broussonin B (64), broussonin F (65), 2'-deoxyadenosine (66), thymidine (67), 2'-deoxyuridine (68),^[21,25] δ -tocopherol (69), 3,5,4'-trihydroxy-bibenzyl-3-O- β -D-glucoside (70), {(4R,5S,10S)-8,9,10-trihydroxy-4-[3'-methoxy-4'-hydroxyphenyl]-1,6-dioxaspiro [4,5] decan-2-one} (71) and 4-hydroxyacetophenone (72).^[19-20,25] Their structures are shown in Figure 5.

Figure 5 Chemical structures of other compounds isolated from *B. papyrifera*.

Bioactivities

It is rich in flavonoids and terpenoids in *B. papyrifera*, and these compounds possess widely bioactivities. For example, previous studies revealed that apigenin had anti-tumor, anti-viral, anti-bacterial activities,^[27] alkaloids had cytotoxicity.^[28] Monocyclic monoterpene had the effect of treating prostatitis.^[29-32]

Antioxidant activity

Pang *et al.*^[26] studied the chemical constituents of scorpion fatty oil (FOFB) and its effects on plasma lipids. The main compound 8,11-octadecadienoic acid (50), which was a potent

natural antioxidant in FOFB, inhibited the production of NO by LPS-induced RAW264.7 macrophages.^[33]

Han *et al.*^[34] used ultrasound-assisted extraction to extract *B. papyrifera* fruits (BPP). Then, BPP was purified, and three purified fractions (designated BPP-1, BPP-2 and BPP-3) were obtained for further physicochemical properties. *In vitro* activity revealed that BPP-3 (IC₅₀ = 0.57 µg/mL) and BPP-1 (IC₅₀ = 0.54 µg/mL) had the better antioxidant activity, compared with BPP-2 (IC₅₀ = 0.86 µg/mL).

Antibacterial activity

Li *et al.*^[14] obtained nine compounds from the EtOAc fraction of the mulberry barks. *In vitro* antibacterial test showed that 7,4'-dihydroxy-3'-prenylflavan (**2**) and oleanolic acid (**31**) exhibited stronger antimicrobial activity against *Actinomyces naeslundii* ATCC 12104 (1.95, 1.95 µg/mL) and *Porphyromonas gingivalis* ATCC 33277 (3.90, 1.95 µg/mL) than the positive control [*A. naeslundii* ATCC 12104 (3.9 µg/mL); *P. gingivalis* ATCC 33277 (7.80 µg/mL)].

Sohn *et al.*^[35] reported that a prenylated flavonol compound Papyriflavonol A (Pap A) (**10**) was isolated from the mulberry roots, and evaluated its antimicrobial activity. The results revealed that the minimum inhibitory concentration (MIC) of Pap A against *Candida albicans* and *Saccharomyces cerevisiae* was between 10 µg/mL and 25 µg/mL, and its antifungal activity was mediated by its ability to disrupt cell membrane integrity. In addition, Pap A had a lower toxic effect than amphotericin B, and for the tested strains (MIC = 10–25 µg/mL), the hemolysis ratio of human erythrocytes was less than 5%.

Geng *et al.*^[16] reported that flavonols in *B. papyrifera* showed significantly anti-oral microbial activity *in vitro*. Among them, compound 5,7,3',4'-tetrahydroxy-3-methoxy-8,5'-diprenylflavone (**8**) was more effective in suppressing *Actinomyces naeslundii* and *Porphyromonas gingivalis* (MIC = 1.95 ppm) than the positive control, triclosan.

Anti-proliferative activity

Guo *et al.*^[15] isolated and purified six compounds from EtOAc extract of mulberry bark and studied their biological activities. Cell activity revealed that the compounds broussonchalcone A (**6**), 5,7,3',4'-tetrahydroxy-3-methoxy-8,5'-diprenylflavone (**8**), uralenol (**9**), papyriflavonol A (**10**), and broussonchalcone B (**11**) showed significant anti-proliferative effects on ER-positive breast cancer MCF-7 cells *in vitro*, and the compounds 5,7,3',4'-tetrahydroxy-3-methoxy-8,5'-diprenylflavone (IC₅₀ = 4.41 µM) and broussonchalcone B (IC₅₀ = 4.19 µM) were more effective than the positive control, icaritin. In an established human breast cancer BCAP-37 xenograft BALB/c nude mice model, compounds **8** and **11** reduced tumor growth significantly at the concentrations of 1 µM. Both compounds reduced ERK phosphorylation. Western blot indicated the compounds down-regulated strongly expression concentrations of estrogen receptor-α (ER-α).

Liu *et al.*^[36] reported that the effects of flavonoids on the proliferation of mouse epidermal stem cells *in vitro*. The results found that flavonoids inhibited cell proliferation and it was related to the concentration of compounds. When the concentrations of flavonoids were between 5 µg/mL and 10 µg/mL, the inhibition rate of epidermal stem cells reached 50%, and the high correlation between inhibitory effect and dosage of flavonoids on cells at 2 d (*r*_{2d} = -0.826), 4 d (*r*_{4d} = -0.810) and 6 d (*r*_{6d} = -0.750), respectively.

Cytotoxic activity

Ran *et al.*^[20] determined the cytotoxicity of the compounds, which were isolated from the leaves by MTT method. They found that the compounds (+)-pinoresinol-4'-O-β-D-glucopyranosyl-4"-O-β-D-apiofuranoside (**36**), liriiodendrin (**37**), and apigenin-6-C-β-D-glycopyranoside (**24**) had different degrees of inhibitory activity on HepG-2 cell line, with the IC₅₀ at 17.19, 14.56 and 19.53 µg/mL, respectively.

Zhang *et al.*^[24] reported that a new compound altertoxin IV (**57**) together with nine known compounds were isolated from the ethyl acetate extract of a culture of the endophytic fungus *Alternaria species* G7 in *B. papyrifera* by means of bioassay-guided fractionation. The compounds had cytotoxic activities against three cancer cell lines (A549, MG-63 and SMMC-7721), of which the compound 3,4',5'-trihydroxy-5-methoxy-6H-benzo [c] chromen-6-one (**40**) showed significant cytotoxic activity with IC₅₀ values of 1.47, 2.11 and 7.34 µg/mL, respectively. However, the compound altersolanol A (**58**) showed significant cytotoxic activity against cell lines MG-63 (IC₅₀ = 0.53 µg/mL) and SMMC-7721 (IC₅₀ = 2.92 µg/mL).

Enzyme inhibition activity

Ryu *et al.*^[17] showed that twelve polyphenols were isolated from the chloroform extract of the roots of *B. papyrifera*. Among them, the most potent inhibitor, papyriflavonol A (**10**) (IC₅₀ = 2.1 µM) had slightly higher α-glucosidase inhibitory activity than α-glucosidase inhibitor deoxynojirimycin (IC₅₀ = 3.5 µM), and the novel α-glucosidase inhibitors broussonchalcone A (**17**) (IC₅₀ = 27.6 µM), broussonchalcone B (**18**) (IC₅₀ = 33.3 µM) were similar in activity to sugar-derived α-glucosidase inhibitors voglibose (IC₅₀ = 23.4 µM). Further studies by Ryu *et al.*^[37] found that the compound broussonchalcone A (**6**) (IC₅₀ = 5.8 µM) and the compound 3,4-dihydroxyisolonchocarpin (**13**) (IC₅₀ = 7.7 µM) were the major contributors to the inhibition of xanthine oxidase, and the compound broussonchalcone A was more effective.

Lou *et al.*^[18] reported that a new isoprenylated flavonol was isolated from the branches of *B. papyrifera*, and this compound broussonchalcone A (**21**) showed significant inhibitory effect on PTP1B with an IC₅₀ value of 0.83±0.30 µM.

Park *et al.*^[38] evaluated the inhibitory activity of Broussonchalcone A (**6**) against 3-chymotrypsin-like and papain-like coronavirus cysteine proteases. The results showed papyriflavonol A (**10**) was the most potent inhibitor of PL^{pro} with an IC₅₀ value of 3.7 µM.

Conclusion and perspective

B. papyrifera as a high medicinal value plant is widely distributed in China, and its leaves, roots, barks and fruits can be applied in clinical practice. The chemical compositions of *B. papyrifera* mainly including flavonoids and terpenoids are complex and diverse, the diversity of its chemical ingredients leads to a wide range of pharmacological activities, which are antioxidant, antibacterial, anti-proliferative, cytotoxic, enzyme inhibition and other activities. It should be pointed out that the researchers focus mainly on finding compounds with better activity in *B. papyrifera*, but do not conduct in-depth research on the mechanism of their action, which is difficult to explain the material basis of clinical application of *B. papyrifera*, and greatly limits the construction of trees. For more rationally and effectively to develop resources in *B. papyrifera*, researchers should perform further research on its chemical constituents, bioactivities and the mechanism of action in the future.

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