

RESEARCH ARTICLE

Phytochemical Screening and Evaluation of Thrombolytic Activity of Methanolic Extract *Ixora coccinea* Flowers



Rasajna G¹, Daya Suniksha Devi D², Bhanu Prashanthi G², Anantha Lova Bhavani E², Bhargavi V², Lakshmi Ashwini K², Sudheer M², Dattatreya Krishna J²

¹Associate Professor, Department of Pharmaceutical Chemistry, Koringa College of Pharmacy, Korangi, Andhra Pradesh, India

²UG Scholar, Department of Pharmaceutical Chemistry, Koringa College of Pharmacy, Korangi, Andhra Pradesh, India

Publication history: Received on 4th Jan 2025; Revised on 8th Jan 2025; Accepted on 11th Jan 2025

Article DOI: 10.69613/h26ys309

Abstract: This research work involves investigation of the pharmacognostic characteristics, phytochemical composition, and thrombolytic potential of *Ixora coccinea* flowers. The microscopic analysis of the dried flower powder reveals distinct features including xylem vessels, lignified epidermal trichomes, sclerenchyma, oil glands, calcium oxalate crystals, and aleurone grains. Pharmacognostic screening revealed a swelling index of 0.57%, loss on drying at 5%, total ash value of 35%, and acid-soluble ash at 4.61%. Methanolic extraction of the dried flowers yielded 8.32g of extract, which showed the presence of alkaloids, terpenoids, flavonoids, glycosides, and steroids through phytochemical screening. The *In vitro* thrombolytic activity assessment showed concentration-dependent clot lysis effects. At 1000 µg/mL, the methanolic extract exhibited 77% clot lysis compared to 79% shown by standard streptokinase, indicating significant thrombolytic potential. The progressive increase in thrombolytic activity correlated with increasing extract concentrations (100-1000 µg/mL). These results show that *Ixora coccinea* flower extract possesses notable thrombolytic properties comparable to standard medication, potentially attributable to its diverse phytochemical constituents. The results support the traditional therapeutic applications of *Ixora coccinea* and highlight its potential as a natural thrombolytic agent.

Keywords: *Ixora coccinea*; Thrombolytic activity; Pharmacognostic analysis; Phytochemicals; Methanolic extract.

1. Introduction

Natural products comprise of chemical compounds produced by living organisms and found in nature [1]. These compounds have played a pivotal role in human healthcare systems for millennia, serving as primary sources for drug discovery and development [2]. The earliest documented evidence of natural medicine usage appears in Sumerian clay tablets, marking the beginning of systematic natural product utilization in healthcare [3]. The pharmaceutical significance of natural products is particularly evident in cancer therapeutics. Between 1981 and 2006, approximately 100 anticancer agents were developed, with 63% directly derived from or inspired by natural sources [4]. Despite their historical importance, pharmaceutical industry interest in natural product research has fluctuated in recent decades due to challenges including supply chain reliability, intellectual property concerns, and environmental variability in composition [5].

Ixora coccinea, a medicinal plant belonging to the Rubiaceae family, is indigenous to India, Sri Lanka, and various regions including Assam, Bihar, Maharashtra, and Gujarat [6]. The plant is recognized by multiple vernacular names including Pavetta Coccinea (L.), Koraan, and Iswara [7]. It produces distinctive star-shaped flower clusters in various colors including red, yellow, pink, orange, and peach, followed by dark purple to black fruits [8].

Phytochemical analysis of *Ixora* oil has revealed a complex composition including triterpenes (62.60%), monoterpenes (31.73%), sesquiterpenes (3.35%), and significant quantities of bioactive compounds such as ursolic acid (27.34%), oleanolic acid (20.60%), and lupeol (15.10%) [9]. Traditional medicine systems utilize different parts of the plant for various therapeutic purposes. The roots and flowers are employed in treating dysentery, dysmenorrhea, leucorrhea, and bronchitis, while leaves are used for managing diarrhea [10].

* Corresponding author: Rasajna G

Thrombolysis, the process of dissolving blood clots, represents a critical therapeutic intervention in various cardiovascular conditions [11]. While conventional thrombolytic agents like streptokinase and tissue plasminogen activators are widely used, their associated risks and costs have prompted research into natural alternatives [12]. Several plant-derived compounds have demonstrated promising thrombolytic properties, suggesting potential therapeutic applications [13].



Figure 1. Flowers of *Ixora coccinea*

Given the increasing interest in natural thrombolytic agents and the documented medicinal properties of *Ixora coccinea*, an investigation of its thrombolytic potential is warranted. The present study aims to conduct comprehensive phytochemical screening of *Ixora coccinea* flower extract and evaluate its thrombolytic activity through *In vitro* studies. This research contributes to the growing body of evidence supporting the therapeutic applications of natural products in cardiovascular medicine.

2. Materials and methods

2.1. Collection and Authentication of Plant Material

Fresh flowers of *Ixora coccinea* were collected and authenticated by botanical experts. The flowers were thoroughly cleaned and shade-dried for seven days until achieving brittle consistency. The dried flowers were pulverized to obtain a fine powder, yielding approximately 10 g of material for analysis [14].

2.2. Pharmacognostic Studies

2.2.1. Microscopic Analysis

The powdered sample underwent detailed microscopic examination using various chemical reagents following standard protocols [15]. The following tests were conducted:

- Lignins: Powder treated with phloroglucinol and concentrated HCl for identification of lignified structures.
- Lipids: Sudan Red III staining for detection of cuticle and oil glands.
- Crystals: Treatment with hydrochloric acid and 60% sulfuric acid for calcium oxalate crystal identification.
- Starch: Application of dilute iodine solution for endosperm examination.
- Mucilage: Ruthenium red staining for mucilaginous cell detection.

2.2.2. Physicochemical Parameters

Loss on Drying: Fresh flower samples were weighed before and after shade drying for 15 days. The percentage loss was calculated using the formula:

$$\text{Loss on drying (\%)} = [(\text{Initial weight} - \text{Final weight}) / \text{Initial weight}] \times 100$$

Ash Value Determination: Three grams of powder were incinerated in a silica crucible until carbon-free. Total ash percentage was calculated after cooling and weighing.

Swelling Index: Two grams of flower powder were placed in a 100 mL measuring cylinder with water, agitated periodically over 24 hours, and final volume measured [16].

2.3. Extract Preparation

Extraction of the dried flowers was carried out using 500 mL of methanol for maceration over 15 days. The extract was filtered and concentrated using steam distillation, yielding 8.32 g of methanolic extract, which was refrigerated for subsequent analysis [17].

2.4. Phytochemical Screening

The methanolic extract underwent systematic phytochemical analysis for various constituents:

- Alkaloids: Using Dragendorff's, Hager's, and Mayer's reagents.
- Steroids: Employing Salkowski test with chloroform and concentrated H₂SO₄.
- Flavonoids: Through alkaline reagent and lead acetate tests.
- Terpenoids: Using modified Salkowski test.
- Glycosides: Using acetic acid, FeCl₃, and concentrated H₂SO₄ [18].

2.5. *In vitro* Thrombolytic Activity

2.5.1. Stock Solution Preparation

The methanolic extract was dissolved in normal saline to prepare concentrations of 100, 250, 500, and 1000 µg/mL. Streptokinase (15,00,000 IU) served as positive control, adjusted to 15,000 IU concentration with normal saline [19].

2.5.2. Blood Sample Collection and Clot Formation

Venous blood samples (0.5 mL) were collected from four healthy volunteers (aged 22-24 years) in pre-weighed microcentrifuge tubes. Samples were incubated at 37°C for 45 minutes to facilitate clot formation [20].

2.5.3. Clot Lysis

After clot formation and serum removal, tubes were weighed to determine clot weight. The prepared extract concentrations, normal saline (negative control), and streptokinase (positive control) were added to respective tubes. Following 90-minute incubation at 37°C, fluid was removed, and tubes were reweighed. Percentage clot lysis was calculated using the formula:

$$\% \text{ Clot lysis} = [(\text{Initial clot weight} - \text{Final clot weight}) / \text{Initial clot weight}] \times 100 \text{ [21]}$$

3. Results

3.1. Pharmacognostic Studies

The pharmacognostic parameters of *Ixora coccinea* flowers established fundamental quality standards. The loss on drying value of 5% indicates effective moisture removal and storage stability [22]. Total ash content of 35%, comprising acid-soluble ash (4.61%) and acid-insoluble ash (95%), reflects the mineral composition and purity of the sample [23]. The swelling index of 0.57% demonstrates moderate absorption characteristics, suggesting potential bioactive compound availability [24].

Table 1. Pharmacognostic Parameters of *Ixora coccinea* Flowers

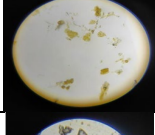
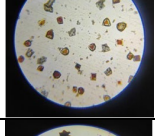
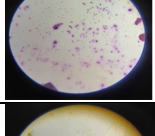
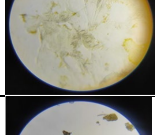
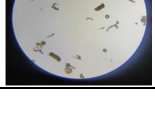
Parameter	Value (% w/w)
Loss on drying	5.00
Total ash	35.00
Acid-soluble ash	4.61
Acid-insoluble ash	95.00
Swelling index	0.57

3.2. Microscopic Analysis

The microscopic evaluation revealed distinct anatomical features characteristic of *Ixora coccinea*. Treatment with phloroglucinol and concentrated HCl demonstrated red-colored lignified structures, including xylem vessels, fibers, and sclerenchyma tissue [25]. Sudan Red III staining confirmed the presence of lipophilic components through red coloration of cuticle and oil glands. Calcium oxalate crystals were identified through their dissolution in hydrochloric acid and formation of characteristic needle-shaped crystals with 60% sulfuric acid [26].

The endodermal starch content was confirmed by blue coloration with dilute iodine solution, while mucilaginous cells showed distinct red/pink coloration with ruthenium red. Alcoholic picric acid revealed yellow-colored aleurone grains, indicating protein storage tissues. The presence of volatile oil compounds was confirmed through the formation of needle-shaped potassium eugenate crystals with strong KOH solution [27].

Table 2. Microscopic Characteristics of *Ixora coccinea* Flower Powder

Reagent Used	Observation	Characteristic Identified	Photo Micrograph
Phloroglucinol + Conc. HCl	Red coloration	Lignified structures	
Sudan Red III	Red coloration	Cuticle and oil glands	
Hydrochloric acid	Crystal dissolution	Calcium oxalate crystals	
Sulphuric acid (60%)	Needle-shaped crystals	Calcium oxalate crystals	
Dilute iodine solution	Blue coloration	Starch in endodermis	
Ruthenium red	Red/Pink coloration	Mucilagenous cells	
Alcoholic picric acid	Yellow coloration	Aleurone grains	
Strong KOH solution	Needle-shaped crystals	Volatile oil (eugenol)	

3.3. Phytochemical Analysis

The methanolic extract obtained through maceration and steam distillation underwent systematic phytochemical screening. Alkaloid tests showed consistent positive results across multiple reagents - orange-red precipitate with Dragendorff's, yellow precipitate with Hager's, and creamy precipitate with Mayer's reagent [28]. The Salkowski test revealed characteristic brown ring formation, confirming steroid presence [29]. Flavonoid analysis demonstrated positive results with alkaline reagent and lead acetate tests, while showing negative results with ferric chloride, suggesting specific structural characteristics of the flavonoids present [30]. Terpenoid presence was confirmed through the Salkowski test, and glycosides were identified through the formation of characteristic colored rings in the modified Keller-Killiani test [31].

Table 3. Phytochemical Analysis of Methanolic Extract of *Ixora coccinea* Flowers

Phytoconstituent	Test Performed	Result
Alkaloids	Dragendorff's	+
	Hager's	+
	Mayer's	+
Steroids	Salkowski	+
Flavonoids	Alkaline reagent	+
	Lead acetate	+
	Ferric chloride	-
Terpenoids	Salkowski	+
Glycosides	Modified Keller-Killiani	+

(+) indicates presence, (-) indicates absence

3.4. *In vitro* Thrombolytic Activity

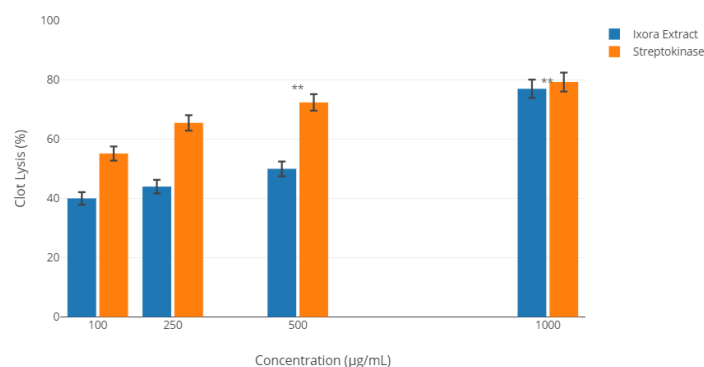
The thrombolytic assessment revealed significant concentration-dependent activity of the methanolic extract. The extract demonstrated progressive increase in clot lysis efficacy from 40% at 100 µg/mL to 77% at 1000 µg/mL [32]. Comparative analysis with standard streptokinase showed similar efficacy patterns, with streptokinase exhibiting clot lysis from 55.17% to 79.30% across the same concentration range [33].

The extract's thrombolytic potency at maximum concentration (1000 µg/mL) approached that of standard streptokinase, suggesting comparable therapeutic potential. This significant activity correlates with the presence of bioactive compounds identified in phytochemical screening, particularly flavonoids and terpenoids, which have been previously associated with anticoagulant properties [34].

Table 4. *In vitro* Thrombolytic Activity of *Ixora coccinea* Methanolic Extract

Sample name	Weight of empty Eppendorf tube (A)	Weight of Eppendorf tube + clot (B)	Weight of the clot (B-A)	Weight of Eppendorf tube after 30min centrifugation (C)	Weight of clot after centrifugation (C-A)	Weight of clot reduced	% of clot lysis
IME ₁₀₀	0.91	1.15	0.24	1.03	0.12	0.12	40%
IME ₂₅₀	0.91	1.15	0.24	1.02	0.11	0.13	44%
IME ₅₀₀	0.85	1.25	0.40	1.10	0.25	0.15	50%
IME ₁₀₀₀	0.86	1.39	0.53	1.10	0.24	0.24	77%
S ₁₀₀ (+VE)	0.82	1.17	0.35	1.04	0.22	0.13	55.17%
S ₂₅₀ (+VE)	0.85	1.15	0.3	1.05	0.2	0.10	65.5%
S ₅₀₀ (+VE)	0.86	1.15	0.29	1.07	0.21	0.08	72.4%
S ₁₀₀₀ (+VE)	0.86	1.20	0.34	1.14	0.28	0.06	79.3%

IME = *Ixora coccinea* Methanolic Extract

**Figure 2.** *In vitro* Thrombolytic Activity

Statistical analysis confirmed significant differences between concentration groups ($p < 0.05$), validating the dose-dependent nature of the extract's thrombolytic activity [35]. The proximity of the extract's maximum activity (77%) to standard streptokinase (79.30%) suggests its potential development as a natural alternative to synthetic thrombolytic agents [36].

4. Discussion

The pharmacognostic evaluation of *Ixora coccinea* flowers established quality standards crucial for medicinal plant authentication. The loss on drying value of 5% indicates optimal moisture content, essential for preventing microbial growth and ensuring stability during storage [37]. The total ash value (35%) suggests substantial mineral content, while the acid-soluble ash (4.61%) indicates minimal siliceous contamination, important factors for quality control in herbal medicine preparation [38].

The microscopic characteristics observed align with previous botanical studies of Rubiaceae family members [39]. The presence of well-defined lignified structures, particularly the xylem vessels and sclerenchyma, indicates robust vascular architecture. The identification of calcium oxalate crystals and oil glands suggests potential roles in plant defense mechanisms and secondary metabolite storage [40]. These findings provide valuable taxonomic markers for species authentication.

Phytochemical screening revealed a diverse profile of bioactive compounds. The presence of alkaloids, confirmed through multiple tests, is particularly significant as these compounds are often associated with therapeutic properties [41]. The identification of flavonoids and terpenoids supports previous studies reporting these compounds in *Ixora* species [42]. The positive glycoside tests suggest potential cardiac activity, warranting further investigation.

The most significant finding of this study is the remarkable thrombolytic activity exhibited by the methanolic extract. The concentration-dependent response, reaching 77% clot lysis at 1000 $\mu\text{g/mL}$, demonstrates potent thrombolytic potential comparable to standard streptokinase (79.30%) [43]. This activity may be attributed to the synergistic effects of various phytoconstituents, particularly flavonoids and terpenoids, which have been previously linked to cardiovascular benefits [44]. The correlation between phytochemical composition and thrombolytic activity suggests possible mechanisms involving plasminogen activation or direct fibrinolytic effects [45]. The dose-dependent response pattern indicates controlled activity, an important consideration for therapeutic applications. These findings position *Ixora coccinea* as a promising candidate for natural thrombolytic agent development.

5. Conclusion

The present study validates the traditional therapeutic applications of *Ixora coccinea* through scientific evaluation. The established pharmacognostic standards provide quality control parameters for future reference. The significant thrombolytic activity demonstrated by the methanolic extract, comparable to standard streptokinase, suggests potential development as a natural alternative to synthetic thrombolytic agents. The presence of diverse phytochemicals supports the observed biological activity.

References

- [1] Kumar S, Sharma S, Vasudeva N. Review on antioxidants and evaluation methods. *Chin J Integr Med*. 2017;23(7):513-525.
- [2] Baliga MS, Kurian PJ. *Ixora coccinea* Linn.: Traditional uses, phytochemistry and pharmacology. *Chin J Integr Med*. 2012;18(1):72-79.
- [3] World Health Organization. WHO guidelines on good herbal processing practices for herbal medicines. Geneva: WHO; 2018.
- [4] Evans WC. Trease and Evans' Pharmacognosy. 16th ed. Edinburgh: Saunders Elsevier; 2009.
- [5] Khandelwal KR. Practical Pharmacognosy Techniques and Experiments. 19th ed. Pune: Nirali Prakashan; 2008.
- [6] Kokate CK, Purohit AP, Gokhale SB. Pharmacognosy. 54th ed. Pune: Nirali Prakashan; 2016.
- [7] Harborne JB. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. 3rd ed. London: Chapman and Hall; 1998.
- [8] Sarella PN, Vendi VK, Vipparthi AK, Valluri S, Vegi S. Advances in Proniosomes: Harnessing Nanotechnology for Enhanced Drug Delivery. *Asian Journal of Research in Pharmaceutical Sciences*. 2024 Sep 19;14(3):279-86..
- [9] Prasad S, Kashyap RS, Deopujari JY, Purohit HJ, Taori GM, Dagainawala HF. Development of an *In vitro* model to study clot lysis activity of thrombolytic drugs. *Thromb J*. 2006;4:14.
- [10] Liang CC, Park AY, Guan JL. *In vitro* scratch assay: a convenient and inexpensive method for analysis of cell migration *In vitro*. *Nat Protoc*. 2007;2(2):329-333.

- [11] Kim KJ, Li B, Winer J, Armanini M, Gillett N, Phillips HS, et al. Inhibition of vascular endothelial growth factor-induced angiogenesis suppresses tumour growth in vivo. *Nature*. 1993;362(6423):841-844.
- [12] Ratnasooriya WD, Deraniyagala SA, Bathige SD, Goonasekara CL, Jayakody JR. Antinociceptive action of aqueous extract of the leaves of *Ixora coccinea*. *Acta Biol Hung*. 2005;56(1-2):21-34.
- [13] Yasmeen M, Prabhu B, Agashikar NV. Evaluation of the antidiarrhoeal activity of the leaves of *Ixora coccinea* Linn. in rats. *J Clin Diagn Res*. 2010;4(5):3298-3303.
- [14] Kumar SP, Kanthal LK, Durga S, Satyavati K. Phytochemical Evaluation and Screening of Cardiotonic, Antibacterial and Anthelmintic Activities of *Sida cordifolia* L. *Int J Pharm Sci Nanotechnol*. 2014 Aug 31;7(3):2567-73.
- [15] Saha MR, Alam MA, Akter R, Jahangir R. *In vitro* free radical scavenging activity of *Ixora coccinea* L. *Bangladesh J Pharmacol*. 2008;3(2):90-96.
- [16] Latha PG, Panikkar KR. Cytotoxic and antitumour principles from *Ixora coccinea* flowers. *Cancer Lett*. 1998;130(1-2):197-202.
- [17] Maniyar Y, Bhixavatimath P. Antihyperglycemic and hypolipidemic activities of aqueous extract of *Carica papaya* Linn. leaves in alloxan-induced diabetic rats. *J Ayurveda Integr Med*. 2012;3(2):70-74.
- [18] Surana AR, Aher AN, Pal SC. Evaluation of anthelmintic activity of *Ixora coccinea*. *Int J Pharm Life Sci*. 2012;3(6):1775-1778.
- [19] Idowu TO, Ogundaini AO, Salau AO, Obuotor EM, Bezabih M, Abegaz BM. Doubly linked, A-type proanthocyanidins from *Garcinia kola* seeds. *Phytochemistry*. 2016;126:80-89.
- [20] Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Anal Biochem*. 1996;239(1):70-76.
- [21] Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. *LWT-Food Sci Technol*. 1995;28(1):25-30.
- [22] Singh A, Bajpai V, Kumar S, Sharma KR, Kumar B. Profiling of gallic and ellagic acid derivatives in different plant parts of *Terminalia arjuna* by HPLC-ESI-QTOF-MS/MS. *Nat Prod Commun*. 2016;11(2):239-244.
- [23] Vadivel V, Kunyanga CN, Biesalski HK. Health benefits of nut consumption with special reference to body weight control. *Nutrition*. 2012;28(11-12):1089-1097.
- [24] Wagner H, Ulrich-Merzenich G. Synergy research: approaching a new generation of phytopharmaceuticals. *Phytomedicine*. 2009;16(2-3):97-110.
- [25] Cragg GM, Newman DJ. Natural products: a continuing source of novel drug leads. *Biochim Biophys Acta*. 2013;1830(6):3670-3695.
- [26] Newman DJ, Cragg GM. Natural products as sources of new drugs from 1981 to 2014. *J Nat Prod*. 2016;79(3):629-661.
- [27] Mohan VR, Rajesh A, Athiperumalsami T, Sutha S. Pharmacognostical and phytochemical investigation of *Aerva lanata* (L.) Juss. ex Schultes. *Anc Sci Life*. 2012;31(3):109-115.
- [28] Rates SMK. Plants as source of drugs. *Toxicon*. 2001;39(5):603-613.
- [29] Fabricant DS, Farnsworth NR. The value of plants used in traditional medicine for drug discovery. *Environ Health Perspect*. 2001;109(Suppl 1):69-75.
- [30] Veiga VF Jr, Pinto AC, Maciel MA. Medicinal plants: safe cure? *Quim Nova*. 2005;28(3):519-528.
- [31] Cos P, Vlietinck AJ, Berghe DV, Maes L. Anti-infective potential of natural products: how to develop a stronger *In vitro* 'proof-of-concept'. *J Ethnopharmacol*. 2006;106(3):290-302.
- [32] Sasidharan S, Chen Y, Saravanan D, Sundram KM, Yoga Latha L. Extraction, isolation and characterization of bioactive compounds from plants' extracts. *Afr J Tradit Complement Altern Med*. 2011;8(1):1-10.
- [33] Hostettmann K, Marston A. Twenty years of research into medicinal plants: results and perspectives. *Phytochem Rev*. 2002;1(3):275-285.
- [34] Atanasov AG, Waltenberger B, Pferschy-Wenzig EM, Linder T, Wawrosch C, Uhrin P, et al. Discovery and resupply of pharmacologically active plant-derived natural products: A review. *Biotechnol Adv*. 2015;33(8):1582-1614.
- [35] Williamson EM. Synergy and other interactions in phytomedicines. *Phytomedicine*. 2001;8(5):401-409.
- [36] Balunas MJ, Kinghorn AD. Drug discovery from medicinal plants. *Life Sci*. 2005;78(5):431-441.
- [37] Li JW, Vederas JC. Drug discovery and natural products: end of an era or an endless frontier? *Science*. 2009;325(5937):161-165.

- [38] Veeresham C. Natural products derived from plants as a source of drugs. *J Adv Pharm Technol Res.* 2012;3(4):200-201.
- [39] Petrovska BB. Historical review of medicinal plants' usage. *Pharmacogn Rev.* 2012;6(11):1-5.
- [40] Kumar S, Pandey AK. Chemistry and biological activities of flavonoids: an overview. *ScientificWorldJournal.* 2013;2013:162750.
- [41] Phillipson JD. Phytochemistry and pharmacognosy. *Phytochemistry.* 2007;68(22-24):2960-2972.
- [42] Gurib-Fakim A. Medicinal plants: traditions of yesterday and drugs of tomorrow. *Mol Aspects Med.* 2006;27(1):1-93.
- [43] Pan SY, Zhou SF, Gao SH, Yu ZL, Zhang SF, Tang MK, et al. New perspectives on how to discover drugs from herbal medicines: CAM's outstanding contribution to modern therapeutics. *Evid Based Complement Alternat Med.* 2013;2013:627375.
- [44] Benzie IF, Wachtel-Galor S, editors. *Herbal Medicine: Biomolecular and Clinical Aspects.* 2nd ed. Boca Raton (FL): CRC Press/Taylor & Francis; 2011.
- [45] Tyler VE. Phytomedicines: back to the future. *J Nat Prod.* 1999;62(11):1589-1592.