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Identification and pharmacological activities of secondary metabolites of Phytochemicals

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Abstract

Plant secondary metabolites are organic compounds (alkaloids, flavonoids, phenolics, tannins, anthraquinones, saponins, steroids, lignins, terpenes) produced by plant for self-defense during growth and development in order to protect them from harmful agents. As population is increasing and quality of life is reducing couple with outbreak of some diseases that are posing threat on the economy, a lot of medicinal plants have attracted interest of researchers because of the usefulness of these metabolites in pharmaceuticals, food and cosmetic industries. In this review, the pharmacological activities of some medicinal plants that are rich in secondary metabolites were studied, means of identifying and quantifying using spectrophotometry and chromatography techniques were also discussed. However, this will assist to reduce the uses and dependent on the synthetic drug and the onset of the age related diseases.

Keywords: secondary metabolites; pharmacological activities; qualitative and quantitative analysis; techniques.

1. Introduction

Phytochemicals are name from Greek *φυτόν* (phyton) 'plant'. They are chemical compounds naturally found in plant based and consumed as veggies, beverages, fruits, herbs, and spices contributing to positive or negative health effects. Phytochemicals present interesting biological properties like antioxidant, antibacterial, antiviral, anti-parasitic, anti-inflammatory, antidiarrheal activities. They are biologically classified as primary and secondary metabolites. The primary metabolites are carbohydrates, fats, nucleic acids (purines and pyrimidines), amino acids and chlorophyll, *etc*. They are involved in key functions of living organisms such as metabolism, respiration, growth and development. Secondary metabolites (SMs) are organic compounds that are not part of primary metabolites but are found in different part of the plants, that are used to treat, prevent and cure diseases. SMs are triggered during plants growth and development in order to protect them from harmful agents such as insects and microbes. They are also produced during stressful events such as high ultraviolet (UV) irradiation, flooding, pollution, drought, precipitation, and extreme temperatures [1-2]. SMs are induced and distributed randomly in various parts of the specific plants especially during those biotic and abiotic stressful events. They are unique, colorful with different flavor of pleasant and unpleasant aroma and are produced in low quantity. Secondary metabolites have medicinal, environmental, agricultural and industrial applications. Their applications in pharmaceuticals, food and cosmetic industries have improved the quality of life which boost the economic value, reducing the uses of synthetic drug, increase life expectancy and reduce the

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onset of age related diseases such as osteoporosis, susceptible to infection, neurodegeneration, macular degeneration, chronic inflammation, hearing loss (presbycusis), cardiovascular diseases, metabolic syndrome among others.

This review discusses the pharmacological activities of some medicinal plants that are rich in secondary metabolites, the means of identifying and quantifying using spectrophotometry and chromatography techniques.

2. Secondary metabolites

SMs are alkaloids, flavonoids, phenolics, tannins, anthraquinones, saponins, steroids, glycosides, terpenes, *etc*. (**Figure** 1), are an alternative to many if not all synthetic drugs because of cost effectiveness and low toxicity.

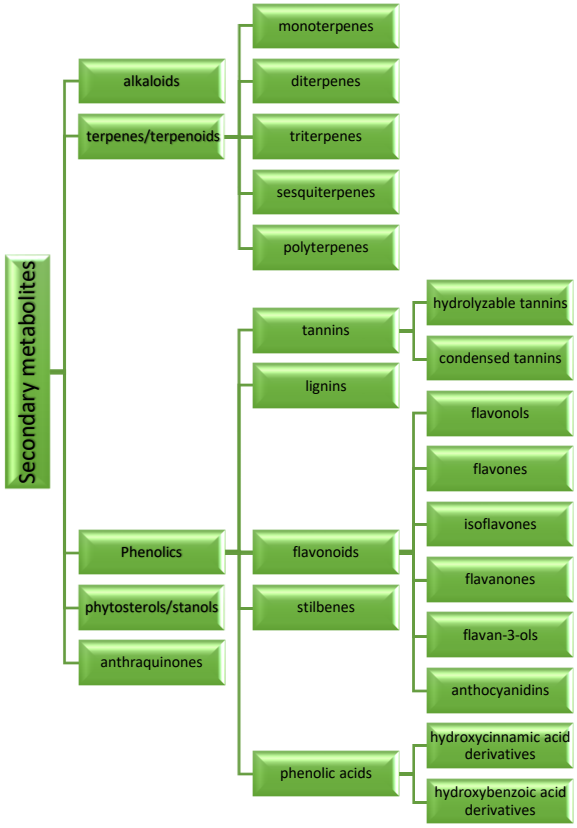

Figure 1. Classification of secondary metabolites

2.1. Alkaloids

Alkaloids are group of phytochemicals that contain at least one nitrogen in a heterocycle. They have bitter taste and other properties of alkali. Some common alkaloids are morphine, ledecorine, strychnine, berberine, atropine, ambinine, rutaecarpine, colchicine, crinamine, lycorine, scopolamine, rauvoxine, cinchonine, ephedrine, harmaline, schumannificine, salsoline, quinine, aricine, nicotine, tetrahydroalstonine, codeine, coniine, scopolamine, hyoscamine, atropine, caffeine, sangunarine, *etc*. (**Figure** 2). They are found in the roots (*Isatis indigotica*, *Stephenia tetrandra*, *Annona muricata* L, *Strychnos panganensis*, *Strychnos matopensis*, *Stemona aphylla*, *Stephania rotunda*); barks (*Alstonia boonei*, *Guatteria hispida*, *Phellodendron chinense*, *Magnolia officinalis*, *Berberis vulgaris*, *Cinchona*

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calisaya, *Zanthoxylum leprieurii* Guill. & Perr., *Schumanniophyton magnificum* HARMS.); stems (*Picrasma quassioides, Esenbeckia leiocarpa*); leaves (*Vernonia amygdalina, Solanum nigrum*, *Nauclea officinalis*, *Pilocarpus microphyllus*, *Rauwolfia vomitoria*); bulbs (*Crinum jagus*, *Crinum glaucum*); seeds (*Hunteria umbrellata*, *Sophora alopecuroides*, *Datura innoxia*, *Datura metel*, *Datura stramonium*, *Cola nitida*, *Cola acuminata*, *Garcinia kola*, *Lupin albus* L., *Centaurea vlachorum*, *Peganum harmala*, *Papaver degenii*, *Erythrina brucei*); and rhizomes (*Sanguinaria canadensis*, *Ligusticum chuanxiong*, *Coptis chinensis*) of many herbaceous and some species of woody plants usually in tropical region. Alkaloids have some biological activity such as antimicrobial, stimulant, analgesic, anthelmintic, anticoagulant, anti-acne, and antioxidant, among others [3-4]. Alkaloids, ambinine and berberine, have been reported to possess antithrombotic and anticoagulant activities [5-6]. Tetramethylpyrazine extracted from *Chuanxiong rhizome* has been used for treatment of Ischemic stroke [7-9]. Quinine extracted from Cinchona tree are used in the treatment of malaria and has served as a source of many other drugs.

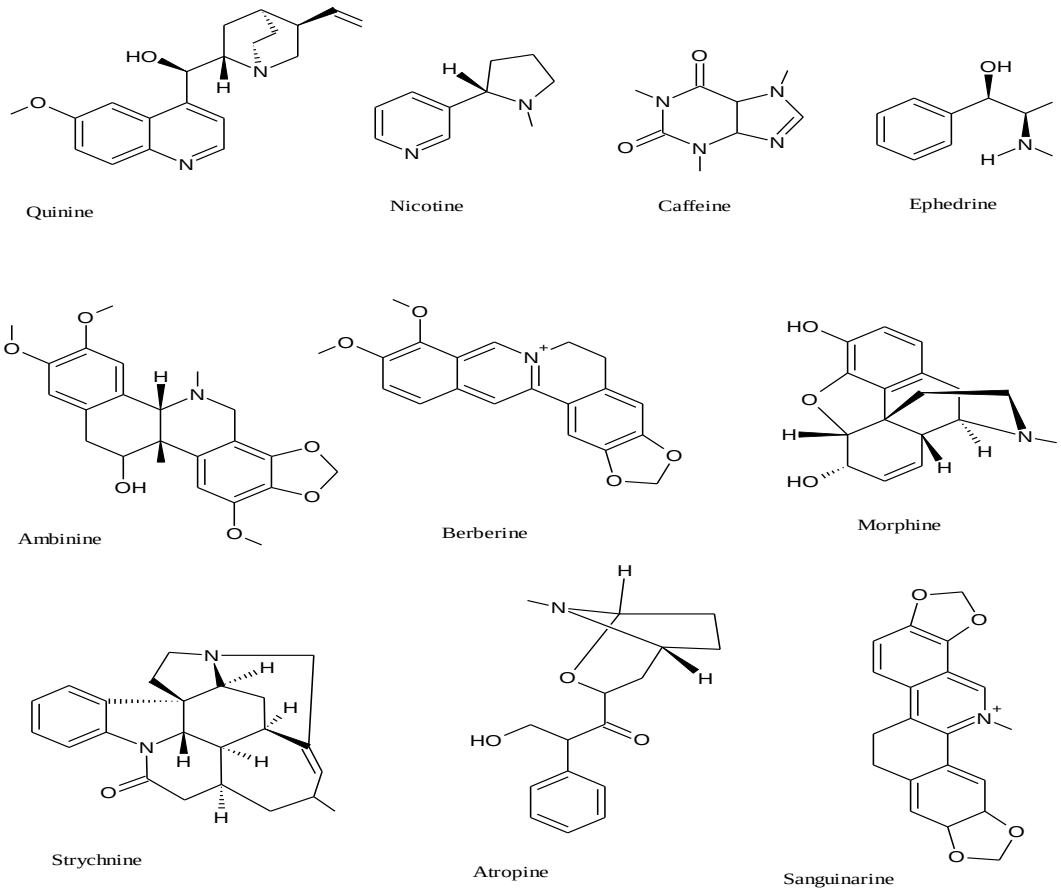

Figure 2. Chemical structure of some alkaloids

2.2. Phenolics

Phenolic compounds are chemical compounds containing one or more aromatic ring with hydroxyl group and other substituents at different positions on the compounds. They are categorized into classes depending on their structure and subcategorized within each class according to what attaches to the ring. Phenolic compounds are classified as

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phenolic acids: hydroxybenzoic and hydroxycinnamic acids (*p*-hydroxybenzoic, ferulic, protocatechuic, vanillic, syringic acids, caffeic, ferulic, chlorogenic acid, *o*-, *m*- and *p*-coumaric acids and sinapic acids); flavonoids: flavonols, flavones, isoflavones, flavanones, flavan-3-ols, and anthocyanidins; tannins: condensed or non-hydrolyzable and hydrolyzable; lignins (arctigenin, arctiin, trachelogenin) and stilbenes (piceatannol, miyabenol, viniferol, hopeaphenol, resveratrol) (**Figure** 3). They are present in flowers, fruits, vegetables, cereals, grains, and seeds. For example, berry (blue, straw, choke, black and cran), avocado, plum, pear, cherry (sweet, sour, black), citrus (orange, grapefruit, lemon), apple, peach, potato, lettuce, spinach, coffee beans, and cider. Phenolic compounds have numerous chemicals, biologicals, agricultural, and medical studies. Many phenolic compounds are used in food, pharmaceutical, and cosmetics production because of their organoleptic properties; color, aroma, taste, and astringency [10]. The biological activities of phenolic compounds are exploited in pharmaceutical industries because of their anti-inflammatory, antioxidant, anti-hypertensive, anti-microbial, hepatoprotective properties, antidiabetic, and anticancer, *etc*. [11]. Arctigenin, arctiin and trachelogenin have been reported as anti-HIV, anticancer [12-13].

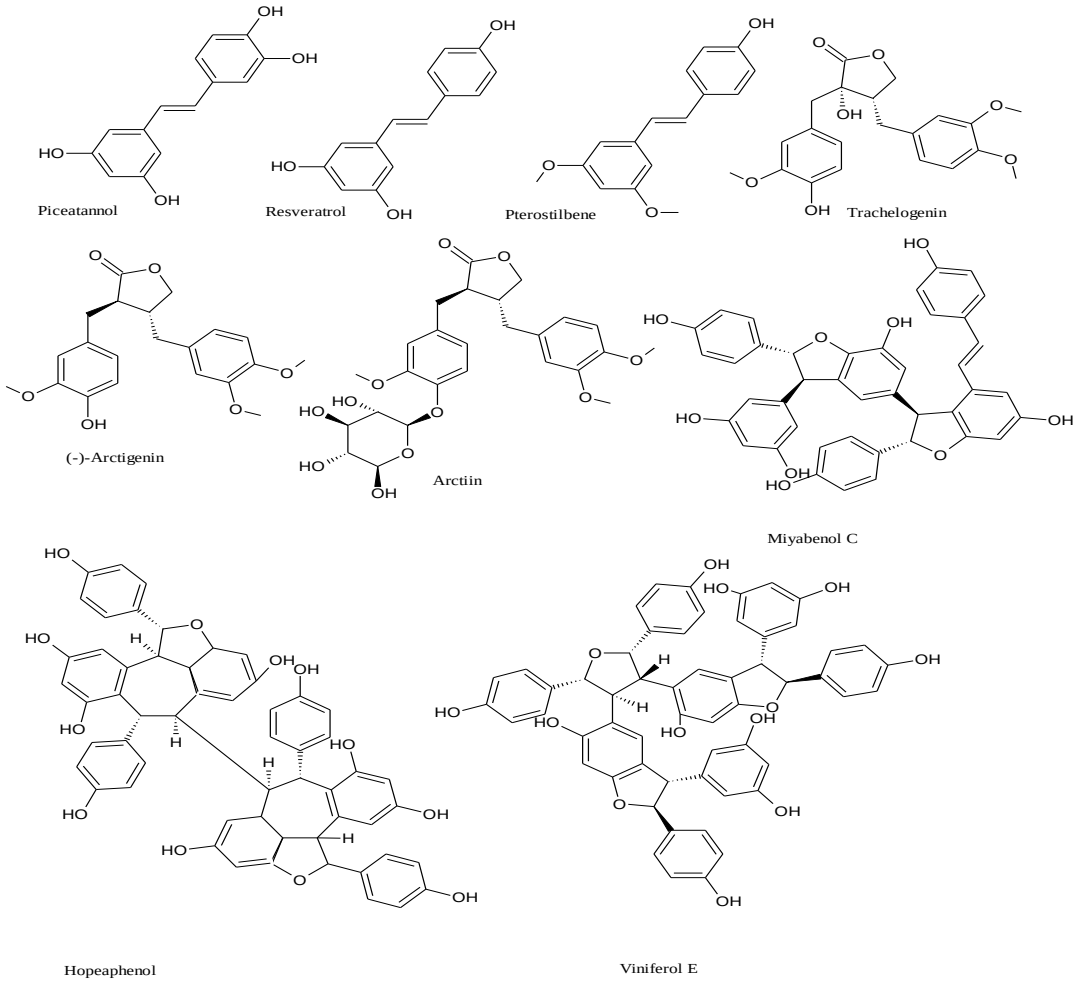

Figure 3. Chemical structures of some lignins and stilbenes

2.3. Flavonoids

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Flavonoids are low molecular weight polyphenols. They are phenolic compounds found in onions, blueberries and other berries, black tea, green tea and oolong tea, bananas, cocoa, all citrus fruits, cannabis, sea-buckthorns, buckwheat, *etc*., with health benefit. Flavonoids have six major subgroups such as flavonols: quercetin, isorhamnetin, kaempferol, myricetin; flavones: fisetin, luteolin, apigenin; isoflavones: genistein, daidzein, glycitein; flavanones: hesperetin, naringenin, eriodictyol; flavan-3-ols: (+)-catechin, (+)-gallocatechin, (-)-epicatechin, (-)-epigallocatechin, (-)-epicatechin-3-gallate, (-)-epigallocatechin-3-gallate, theaflavin, theaflavin-3-gallate, theaflavin-3'-gallate, theaflavin-3,3'-digallate, thearubigins; anthocyanidins: cyanidin, delphinidin, malvidin, pelargonidin, peonidin, petunidin *etc*. (**Figure** 4). Flavonoids have been reported to reduce the risk of all-causing mortality like cardiovascular diseases (CVD), cancer, Parkinson disease (PD) [14-15]. They have been reported to have antioxidant, anti-inflammatory, anti-apoptotic and neuroprotection [16]. Epigallocatechin-3-gallate, epicatechin gallate and gallocatechin-3-gallate have been reported to have antiviral activities such SARS, SARS-CoV, SAR-CoV and SAR-CoV-2 [17-19]. The flavonoids kaempferol, fisetin, quercetin, *etc*. have been shown to spike protein against SAR-CoV-2 where they have low binding free energy with hACE2-S protein complex [20]. Recently, cannflavin A and B have been reported as potent anticancer, anti-inflammatory and neuroprotector [21- 24].

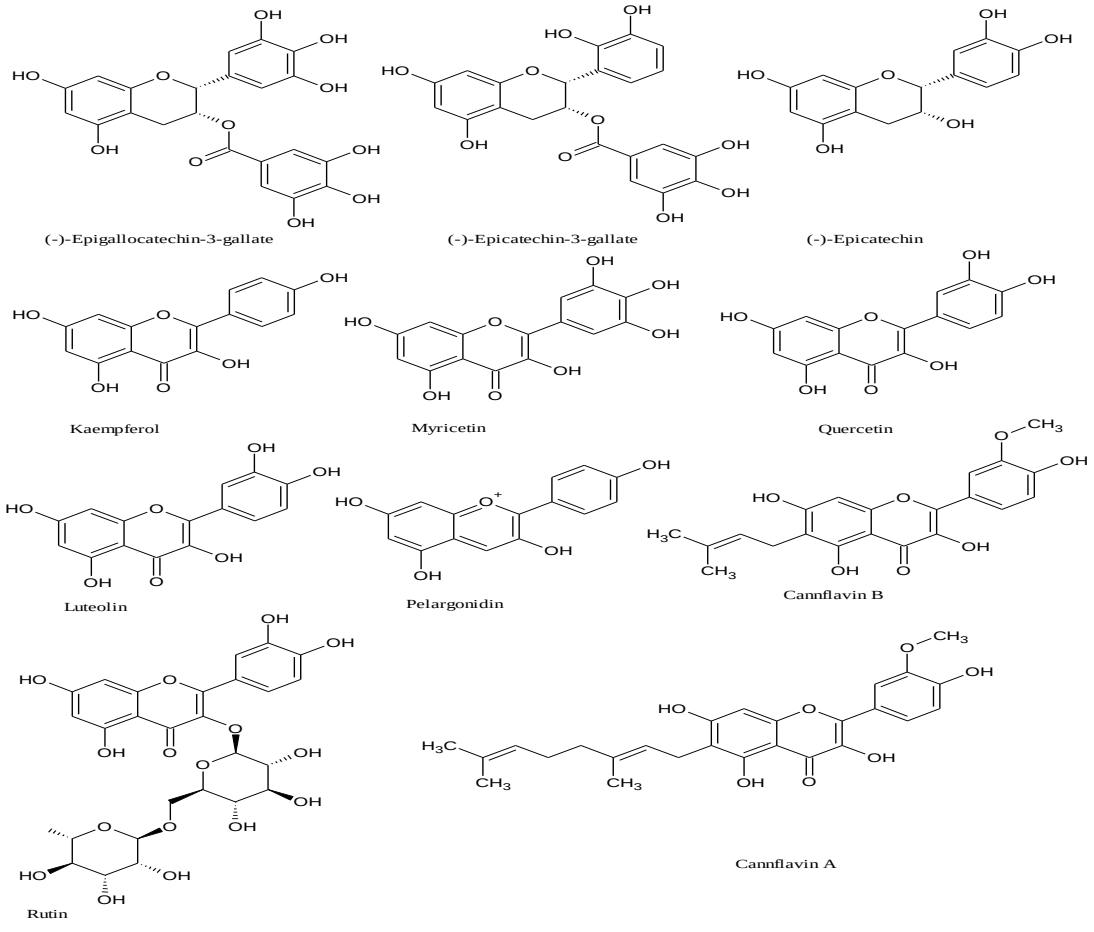

Figure 4. Chemical structure of some flavonoids

2.4. Tannins

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Tannins (or tannic acid) are water-soluble phenolic compounds with a molecular weight greater than 500 Da. They have strong affinities for proteins. Tannins are found in different part of the plant like the bark of trees (*Terminalia arjuna*, *Senna auriculata*, *Casuarina equisetifolia*, *Acacia mearnsii)*; wood (*Sterculia oblonga*, *Castanea sativa*, *Schinopsis balansae*); fruits (*Sideroxylon obtusifolium* (Roem. & Schult.); seeds (*Doliocarpus multiflorus*, *Tetrapterys macrocarpa*, *Acacia nilotica*, *Theobroma cacao*, *Platypodium elegans*); and roots (*Potentilla alba*, *Waldsteinia geoides*, *Casuarina equisetifolia*); leaves (*Camellia sinensis*, *Athrixia phylicoides* L., *Psidium Guajava* L, *Carapa procera* DC., *Nephelium lappaceum* L., *Populus tremuloides*, *Pericopsis laxiflora* (Benth. ex Baker), *Camellia formosensis*); flowers (*Punica granatum*, *Hibiscus Sabdariffa*, L.); buds (*Camellia japonica* L.). Many researchers have found tannins to exhibit potent biological properties such as antioxidant, antibacterial, antiviral, anti-parasitic, anti-inflammatory, antidiarrheal activities etc. [25- 27]. Tannins are classified as non-hydrolyzable (condensed tannin) (catechol-type, proanthocyanidins (procyanidins), profisetinidin, polyflavonoid, pyrocatecollic-type) and hydrolysable (ellagic, acid, camelliins A and B, punicatannin C, punicalin, punicalagin, pedunculagin, gallic acid, and pyrogallic acid) (**Figure** 5). Tannins are used as astringent herbs for hemostatics, gastrointestinal tract, renal, pulmonary, cardiovascular, female reproductive tract and so on. Gallic acid has a wide range of industrial uses: standard for determining phenolic content of analytes in pharmaceutical industry; material for ink, paints and color developer; and in food industry as antioxidants and preservatives.

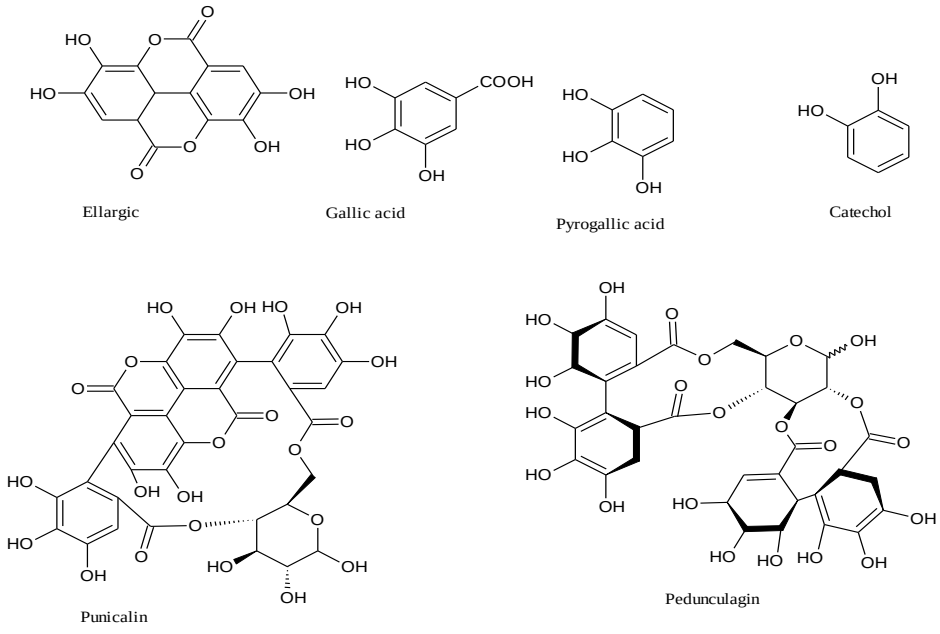

Figure 5. Chemical structure of some tannins

2.5. Terpenoids

Terpenoids are phytochemicals with chemical units of isoprene $(C_5H_8)n$ where n represents the number of isoprenes present in the compound. They are found in spices (*Syzygium aromaticum* L., *Zingiber officinale*, *Thymus vulgaris* L., *Curcuma longa*, *Cinnamomum zeylanicum*, *Cinnamomum cassia*, *Salvia miltiorrhiza Bunge*, *Salvia lavandulaefolia*, *Salvia officinalis*), tea (*Camellia sinensis*), cannabis (*Cannabis sativa*), citrus fruits (*Citrus reticulata*, *Citrus sinensis*,

Citrus aurantium, *Citrus limon*), *Artemisia annua* L., *Ekebergia capensis*, *Salvia divinorum*, *Thapsia garganica*, *Ricinus communis*, *Ginkgo biloba*, *Crocus sativum* L., *etc*. Some examples are of terpenes and terpenoids are limonene, citral, squalene, carotenoids artemisinin, lycopene, curcurbitacin, camphor, and *α*-pinene (**Figure** 6). Different authors have evaluated potential usefulness of terpenes and terpenoids to exhibit anti-inflammatory, antioxidant, antidiabetic, anticancer, geroprotector, and antiatherosclerotic effects [28-29]. *D*-limonene, Linalool, α-pinene, camphor, myrcene, citral, cucurbitacin B have been reported to be active in inflammatory and oxidative stress diseases [29-31]. Carotenoids, are important natural pigments in carrot, tomatoes *etc*., pro-vitamin A are rich sources of antioxidant [32]. Artemisinin, a terpenoid from *Artemisia annua*, is used to treat fever, inflammation, malaria and kidney disorder [33-35].

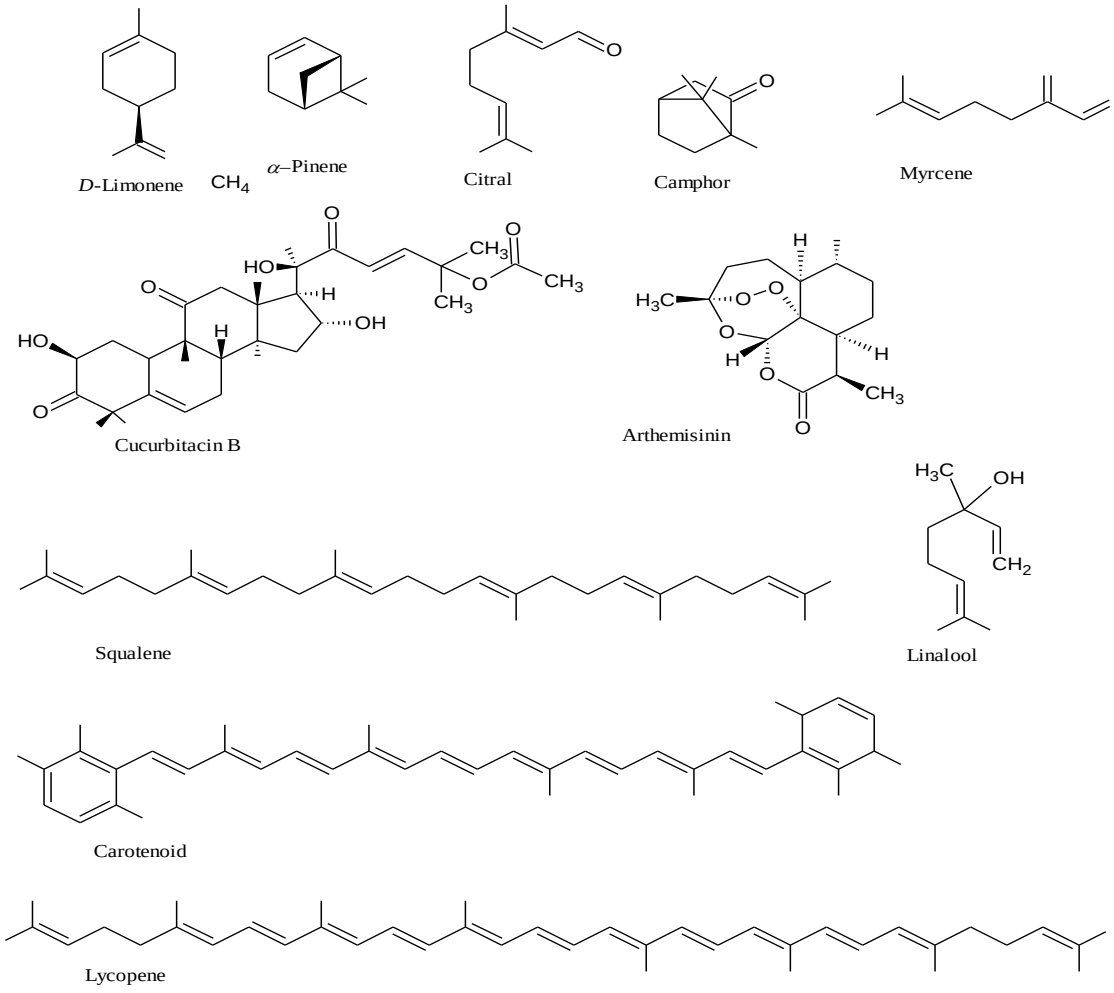

Figure 6. Chemical structure of some flavonoids

2.6. Phytosterols

Phytosterols are esters found in plants but they are similar to cholesterol in animals. They are characterized by tetracyclic cyclopenta-*α*-phenanthrene ring with a specific molecular configuration. Phytosterols are *β*-sitosterol, *β*-

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sitostanol, daucosterol, brassicasterol, campestanol, campesterol, ergosterol, sigmasterol (**Figure** 7). They are found in whole grains, corn oil, wheat germ, rapeseed oil, canola oil, soybeans, green beans, olive oil, sweet potatoes, broccoli, cauliflower, cotton bush (*Asclepias curassavica*), *Grewia tiliaefolia* (GT), *etc*. The plant sterols and stanols have cholesterol-lowering effects in preventing primary and secondary cardiovascular diseases [36-37]. Several researches have been carried to show interesting pharmacological properties as anti-inflammatory, antioxidant, antidiabetic, chemo-preventive, and anti-atherosclerotic effects [38-39].

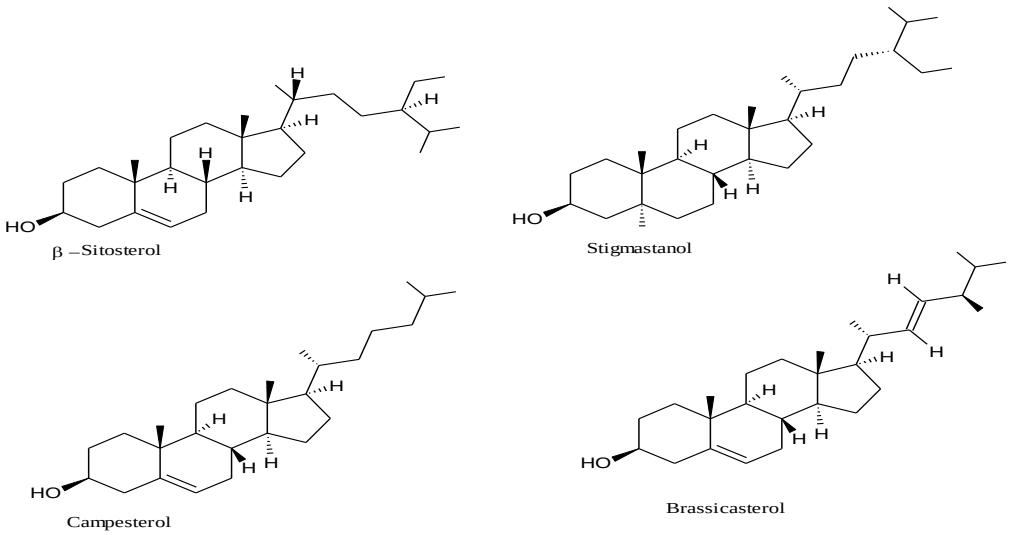

Figure 7. Chemical structure of some phytosterols

3. Pharmacological activities

Phytochemicals (lycopene, lutein, curcumin, resveratrol, perillyl alcohol, *D*-limonene, kaempferol, gingerol, and epigallocatechin-3-gallate etc.), are rich sources of biochemicals, pharmaceuticals and therapeutics agents with little or no toxicity at low doses and of health benefit for treating and preventing most of diseases such as COVID infection [18, 40], coronary heart diseases [41], stroke [6-7], neurodegenerative disorder [24], blood pressure [42-43], osteoporosis [44-46], cancers [47-51], chronic obstructive pulmonary diseases [52-54], diabetes [41, 55-57], diarrheal [58], respiratory problems [59], Human Immunodeficiency Virus (HIV) infection [13, 60-62], mental health problems [24, 63-67] *etc*.

Despite the health benefit of phytochemicals, some plants are toxic (neurotoxic, genotoxic, gastrotoxic, nephrotoxin, renal toxic, hepatotoxic, cytotoxic) even at the lowest doses. Examples are *Ricinus communis*, *Aesculus hippocastanum*, rhubarb, lupines, sanguinolenta, psorospermin, *Nicotina spp*, *Solanum malacoxylon*, *Phaseolus vulgaris*, *Solanum dulcamara*, *Datura stramonium* [61, 68-71], and some are antinutrients such as glycol-alkaloids and cyanogenic glycosides, lupins, phytohaemagglutinin.

4. Qualitative and quantitative analysis of secondary metabolites

Phytochemical analysis is the method of determining organic constituents of the medicinal plants through qualitative and quantitative analysis. The first step in phytochemical analysis is preliminary screening or rather qualitative

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analysis before quantitative analysis. Qualitative analysis detects the presence or absence of a phytochemical in the extracts. It is indicated by color changes and formation of precipitate. Quantitative analysis provides information on the amount or the concentration in the plant extracts. This details information assists in drug discovery, safety assessment of herbal drugs, explanation of the medicinal potentials of plants and determination of the toxicity levels in plants [72].

4.1. Techniques for identify and quantifying secondary metabolites

Modern analytical techniques such as spectrophotometry, chromatography have been used to identify, isolate, characterize and elucidate structural formulas of phytochemicals. The conventional method for identifying and quantifying secondary metabolites is spectrophotometry whereas the non-conventional methods is chromatography. Both techniques have been used to separate, identify and quantify series of structurally different SMs in plants include spectrophotometric [57, 73-75], GC-MS [56, 76], LC-MS [77], LC-MS/MS [56, 76, 78], Liquid Chromatography-High Resolution Electrospray Ionization Mass Spectrometry (LC- HRESIMS) [57], Nuclear Magnetic Resonance (NMR) [44, 79], Ultra-Fast Liquid Chromatography (UFLC-Q-TOF-MS/MS) [80-81], UFLC-triple-Q-TOF-MS/MS [82], Ultra-high performance Liquid Chromatography quadrupole- time of flight mass spectrometry [83], UHPLC-LTQ-Orbitrap [84], UHPLC/Q- Orbitrap-MS [85], HPLC-DAD [86], UHPLC-Q-TOF-MS [87], UHPLC-Q-TOF-MS/MS [88], HPLC-DAD-ESI-MS [89], UPLC-QTOF/MSE [90], UHPLC-MS/MS [91], HPLC-ESI-QTOF-MS [92], UPLC-QTOF-MS/MS [93], UHPLC-Q-TOF-MS/MS [94-95], UHPLC-ESI-MS/MS [96], UHPLC-ESI-Orbitrap-MS/MS [97], UPLC-IT-MS [98], UPLC-Q-TOF-MS [98], UPLC-PDA [45], HPLC-ESI-MS and MS^2 [99], reverse phase high performance liquid chromatography-diode array detector (RP-HPLC-DAD) [74], HPLC-HRMS [100].

4.2. Identification of Secondary metabolites

4.2.1. Flavonoids

4.2.1.1. Add $FeCl_3$ to the plant extract changes from yellow to purple, blackish-red and greenish-blue precipitate [101-103].

4.2.1.2. Shinoda Test: Alcoholic plant extract, magnesium ribbon or powder and conc. HCl turn pink to red color showed the presence of flavonoid [104-106]. The orange to red color indicate flavones, red to crimson indicate flavonoids, crimson to magenta indicate flavonones.

4.2.1.3. The plant extract and conc. HCl turn red color showed the presence of flavonoid [107].

4.2.1.4. Shibata Test: Alcoholic plant extract, metallic magnesium and conc. HCl turn orange to red color. The orange and red color indicate flavones and flavonols respectively [108-109].

4.2.1.5. Pew's Test: Aqueous plant extract, metallic zinc and conc. H_2SO_4 turn red color showed the presence of flavonols [108].

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4.2.1.6. Sodium hydroxide test: Aqueous plant extract and different concentration of sodium hydroxide solution has been used to produce a yellow color that turn colorless on addition of dilute hydrochloric acid confirm flavonoid [102, 104-106, 110-111], and yellow to red color showed the presence of tannin [100-101]. When it produces an emulsion is hydrolyzable tannins.

4.2.2. Test for Tannin

4.2.2.1. Braymer's test: Add $FeCl_3$ solution to the plant extract filtrate or crude to give dark green or bluish black precipitate in of tannin catechol or gallic tannin respectively [79, 102, 104-106, 108, 111-113].

4.2.2.2. Crude plant extract and acetic acid give red color showed the presence of tannin.

4.2.2.3. Lead acetate test: Diluted plant extract and lead acetate to give black-green colored precipitate formed revealed the presence of tannins [104]. The yellow precipitate indicates flavonoid [105, 111, 112].

4.2.2.4. Bromine water Test. A filtrate of alcoholic plant extract and bromine water to produce buff color precipitate an indication of condensed tannins meanwhile hydrolyzable tannins decolorize [101, 114].

4.2.3. Test for cardiac glycosides

4.2.3.1. Keller-Killani Test: A crude plant extract filtrate, glacial CH_3COOH, $FeCl_3$ solution mix and conc. H_2SO_4 to produce reddish-brown color which turns blue on standing, the acetic layer indicates cardiac glycosides of deoxy sugar moiety [102, 110].

4.2.3.2. Kedee's Test: This method determines cardenolides. The dried plant extract, methanolic KOH and alcoholic 3,5-dinitrobenzoic acid heated to produce purple-violet colour that faded gradually through reddish-brown to brownish-yellow with whitish precipitation indicates the presence of lactone ring [103, 105].

4.2.3.3. Legal's test: The crude plant extract, pyridine, sodium nitroprusside and NaOH solution to produce pink to red color that change to colorless [105].

4.2.3.4. Test for Anthraquinones (Borntrager's test): The test involves alkalization of plant extract with an alkaline usually NH_3 or KOH to produce the corresponding phenolate ion. crude or filtrate of plant extract with 10% ammonia solution shaken vigorously for 30 s to produce pink [108], or red color [101, 109], in aqueous layer indicates the presence of free anthraquinones. In the case of glycoside, filtrate of hydrolysable plant extract should be used to identify the anthraquinones.

4.2.3.5. Modified Borntrager's Test: Plant extract, $FeCl_3$ solution, boil and cool, add benzene, and add NH_3 to the organic layer. mix, to produce a rose pink to blood red colored solution [115].

4.2.4. Test for Phytosterols

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4.2.4.1. Liebermann-Bourchard's or acetic anhydride Test: The aqueous crude extracts, $(CH_3CO)_2O$ heating and cooling and then with conc. H_2SO_4 mixed gives a color changes from violet to blue/bluish green confirm steroid core [79, 102, 104-105, 107, 112], but when pink or reddish color appears it indicate triterpene [79, 105, 116].

4.2.4.2. The aqueous crude extracts, chloroform with conc. H_2SO_4 mixed with pink ring or red at the bottom chloroform layer [114].

4.2.4.3. Salkowski's Test: The filtrate of aqueous crude extracts, chloroform with conc. H_2SO_4 mixed with bluish red to purple color in chloroform layer indicates the presence of steroidal aglycone part of the glycoside and red brown to dark brown at the bottom layer indicates triterpenoids [111].

4.2.4. Test for Terpenoids

4.2.5.1. Add CCl_3COOH to crude plant extract with red precipitate an indicative of the presence of triterpenoids [110].

4.2.5.2. The crude extracts, chloroform, conc. $H2SO_4$ mixed with reddish brown ring indicates the presence of triterpene [102, 107, 112].

4.2.5.3. Aqueous crude plant extract and copper acetate solution heated to emerald green colour indicate diterpenes [117].

4.2.6. Test for alkaloids

4.2.6.1. Dragendorff reagent, (0.85 g $Bi(NO_3)_3.5H_2O$ in 40 mL distilled water, 10 mL CH_3COOH, 8 g KI in 20 mL distilled water), is added to the filtrate of acidified extract to form an orange red precipitate [104, 109-110, 118].

4.2.6.2. Mayer's reagent, (1.36 g HgCl2 in 60 mL distilled water, 5 g KI in 10 mL distilled water, 30 mL H_2O_2, is added to the filtrate of acidified extract to form a yellowish or white precipitate [105, 110, 112].

4.2.6.3. Wagner's reagent, (1.27 g I_2, 2 g KI in 100 mL distilled water), is added to the filtrate of acidified extract to form an orange [79], or reddish-brown precipitate [111, 119].

4.2.6.4 Picric acid test: The filtrate of plant extract and picric acid solution produce yellow color precipitate indicate an alkaloid [102, 110, 120].

4.2.6.5 Bouchardat's test: Acidified filtrate of the extract and Boucharat's reagent (dilute iodine solution) to produce reddish brown colour indicate an alkaloid [119].

4.2.6.6 Tannic acid test: Acidified crude plant extract and tannic acid produce yellow color precipitate indicate an alkaloid [120].

4.2.7. Test for Saponin

4.2.7.1. Foam test: Add or no distilled water to crude plant extract shake vigorously and the soapy appearance [102], foam [79, 105], or honeycomb froth [108, 111-112] showed the presence of saponins glycosides.

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4.2.7.2. Frothing test: Add distilled water to aqueous plant extract shake vigorously and heat with the appearance of frothing showed the presence of saponins [107, 115].

4.2.7.3. Olive oil test: Aqueous crude plant extract mixed well with olive oil shake vigorously and the appearance of foam showed the presence of saponins [102, 114].

4.2.7.4. Na_2CO_3 test: Add aqueous plant extract to Na_2CO_3 solution and distilled water shake vigorously with the appearance of foam show the presence of saponins [110].

4.3. Quantification of Secondary metabolites

4.3.1. Test for total phenols

4.3.1.1. Test for total phenols using Folin-Ciocalteu reagent (FCR) method

This method determines the total phenolic compound in plant crude extract where the Folin-Ciocalteu reagent (2.5 g $Na_2MoO_4.2H_2O$, 10 g $Na_2WO_4.2H_2O$ in 70 mL distilled H_2O, then 5 mL 85% H_3PO_4 and 10 mL HCl), a mixture of phosphomolybdate and phosphotungstate reagents ($H_3PMo_{12}O_{40}$ and $H_3PW_{12}O_{40}$) and aluminum chloride ($AlCl_3$), in the presence of phenolics and carried out in alkaline medium Na_2CO_3 to produce a blue color molybdenum tungsten (Mo_8O_{23} and W_8O_{23}) that can be measured in the range of 720-765 nm in visible spectrophotometer against the standard gallic acid. Also, some other standards including quercetin, ferulic acid and caffeic acids have been reported [57, 74, 121]. In this method different ratio of diluted crude extract and Folin-Ciocalteu reagent have been used. Likewise, the incubation has been carried out at room temperature or heated [57, 73-75].

4.3.2. Test for total flavonoids

4.3.2.1. Test for total flavonoids using aluminum chloride colorimetric assay

Total flavonoid content might involve the nitration of aromatic ring of a catechol with its three or four positions not sterically hinder. The reaction form aluminum complexes with carbonyl and hydroxyl groups of flavones and flavonols respectively. Equivalent amount of aqueous crude extract and diluted with or without $NaNO_2$ solution, will be mixed at room temperature, then add to $AlCl_3$ solution mix and neutralized with or without NaOH solution and read the aluminum complex absorbance in the visible spectrophotometer against either catechin, rutin, or quercetin standard. [57, 74-75, 121-122].

4.3.2.2. Test for total flavonoids by gravimetric

Flavonoid has been quantified when plant extract filtrate is evaporated to dryness to obtain a constant weigh [101-102].

4.3.3. Total tannin content

Plant extract filtrate, $FeCl_3$, and potassium ferricyanide ($K_3[Fe(CN)_6]$) in HCl are mixed to show a blue color or read the absorbance against gallic acid [101-102].

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4.3.4. Total alkaloid

4.3.4.1. Total alkaloid using colorimetric

The plant aqueous extract is dissolved in dimethyl sulphoxide (DMSO), diluted with HCl, the filtrate is mixed with bromocresol green solution and phosphate buffer then measure in the range of visible spectrophotometer against the standard atropine [123].

4.3.4.2. Total alkaloid by gravimetric

Acetic acid in ethanol is added to the crude extract, incubate, concentrate and basified with conc. NH_4OH dropwise to get a precipitate, washed with dilute NH_4OH. The filtrate is dried and weighed [102].

4.3.5. Total saponins

Diethylether is added to concentrated plant extract filtrate, the aqueous layer is washed with alcohol (*n*-butanol) and NaCl solution, then evaporate to a constant weigh [102].

4.3.6. Total terpenoid

Extract the filtrate of plant extract with petroleum ether [102].

4.3.7. Total cardiac glycosides using colorimetric

Baljet reagent (95 mL of picric acid at 1% + 5 mL 10% NaOH) is added to dilute plant extract, incubate to produce a yellow to orange measure in the range of visible spectrophotometer against the standard peruvoside [73, 75].

Conclusion

This review has revealed the recent pharmacological activities and has detailed how the plant secondary metabolites can be identified and quantified. However, this will help in the level of new drug discovery and development from the old ones and new secondary metabolites plant based that are safer and effective therapeutic agents in order to improve the quality of life which will boost the economic and reduces the uses and dependent on the synthetic drug. Also, increases life expectancy and reduces the onset of age related diseases.

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