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Phytochemical Review on *Ocimum sanctum, Zingiber officinale, Rosamarinus officinalis* and *Eucalypus globules* for their antitussive and antioxidant activities

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ABSTRACT

In this present investigation, four different plants species including Ocimum sanctum, Zingiber officinale, Rosamarinus officinalis and Eucalypus globules were reviewed based on their considerable antioxidant, antitussive and expectorant properties. The average of total phenolic content was found to be 3.65 ±1.5 mgGAE/gram; 17±12.3mgGAE/gram, 53.3±14.7mgGAE/gram and 102.75±57.4 mgGAE/gram while the average of total flavonoid content was found to be 0.205mgQE/gram; 4.18±1.9 mgQE/gram; 22.74±7.7 mgQE/gram and 35.03±1.03 mgQE/gram of dried weight O. sanctum, Z. officinale, R. officinalis and E. globules, respectively. The compounds such as rosmarinic acid, ursolic acid, chlorogenic acid, zingiberene, camphene etc., were considered for their metabolites having an antitussive and antioxidant activities compounds of these plants. From these findings, we are in process to formulate new antitussive and expectorant formulation from these plant species either in their combination or as crude drugs or particular fractions obtained using adequate standardized methods.

Key words: Medicinal plants, chemical standardization, antioxidant, antitussive activity

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INTRODUCTION

Since ancient time herbal medicine is playing crucial roles in treatment of human diseases with limit side effects. The scientific evidences on safety and efficacy were recorded for various raw material plants and many of exiting herbal modern formulations. Medicinal plants constitute the main source of new pharmaceuticals and healthcare products. A great number of medicinal plants have been used for management of antitussive and other respiratory disorders **[1-4]**. Beside nutritional value, *O. sanctum, Z. officinale*, *R. officinalis* and *E. globules* were reported to have direct or indirect antitussive and expectorant activities **[3, 5]**. Various extracts and natural compounds from these plants have been reported for resolving phlegm, relieving cough, and dispelling wind-cold syndrome **[6-9]**.

The aerial parts of *O. sanctum* (*Labiatae* family) have been used in several traditional medicine systems to cure various diseases **[10]**. *O. sanctum* is important constituent of many Ayurvedic cough syrups and expectorants. It helps to mobilize mucus in bronchitis, sore throat and asthma **[11-14]**. In addition, antimicrobial, antioxidant, anticatarrhal, antispasmodic, anthelminthic, anti-inflammatory, immunomodulator, anti-stress, adaptogenic, cardioprotective, antiulcer, and anti-diabetic activities of extracts and chemical constituent of *O. sanctum* were reported **[15-18]**. The advantage of this plant is that it is traditionally acceptable and considered as safe**[19]**.

However, *O. sanctum* shown to have antifertility and abortificient action in animal studies and it should be there for used cautiously in pregnant women **[20]**.

The rhizomes *Z. officinale* (*Zingiberaceae* family) have been widely used as spices or condiments, eaten raw or cooked as vegetables and used for flavoring food **[21]**. The plant has been used extensively for cold-induced disease, nausea, asthma, cough, colic, heart palpitation, swellings, dyspepsia, loss of appetite, headaches and rheumatism **[22, 23]**. Skin-lightening cosmeceutical products have been developed from rhizomes of ginger.

R. officinalis (Lamiaceae family) is widely known for its numerous applications in the field of food but also for an increasing interest in its health promoting properties. Among the spices with reported antioxidant properties, *R. officinalis* has been used widely in food applications**[24, 25]**. Ethanol and aqueous extracts of *R. officinalis* leaves are used as coleretic, colagogue, hepatoprotective, and antioxidants, but also as light diuretic, antiulcer, antitumor and antiviral products **[26, 27**].

The leaves *E. globulus* Labill (Myrtaceae family) have been used as traditional remedies for the treatment of various disorders such as pulmonary tuberculosis, influenza fungal infections and diabetes **[28, 29]**. In Mexico, extracts were prepared from aerial parts of *E. globules* are used as ingredients of syrups, candies and other remedies to relieve the symptoms of upper respiratory tract infections like cough and sore throat[**30**].

Chemical standardization

The various research works have been conducted on chemical standardization of medicinal plants. The environmental factors have been identified as responsible for changes and determination of the secondary metabolites in a plant **[31-33]**. The total phenolic and flavonoids content may also change due to extraction methods, solvent, age and part of plant material used **[32, 34-37]**. Prashant et al. 2011**[38]** gives an overview of certain extractants and extraction processes with their advantages and disadvantages. Various methods for isolation and quantification of medicinal plant components have been developed including HTPLC, UV-Vis Spectrophotometer, HPLC, HPLC-UV/MS and GC-MS.

Total phenolics and flavonoids content

The total phenolic content of plant extracts are determined spectrophotometrically according to the Folin-Ciocalteu procedure **[39, 40]**. Gallic acid is used as standard and the absorbance is read at 765 nm using UV-Vis spectrophotometer. Total phenolic content is then expressed as mg gallic acid equivalent/g dry or fresh extract (GAEmg/g). The total flavanoid content of plant extracts are determined by Aluminium chloride ($AlCl_3$) colorimetric methods **[39, 41]**. Quercitin is used to generate the standard curve and the absorbance is read at 415 nm using UV-Vis spectrophotometer. Total flavonoid content is then expressed as mg quercitin equivalent/g dry or fresh extract (QE mg/g). The flavonoid content in plant extract could also be expressed with catechin as standard and the absorbance is read at 510 nm using UV-Vis spectrophotometer. Total flavonoids content is then expressed as mg of catechin equivalent (CE) per g of solid of extract **[42]**.

The total phenolic and flavonoids of *O. sanctum* were investigated by various authors **[5, 11, 20, 43]**. The amount of total phenolic content was found to be 3.65mg/1g of plant material while the total flavonoids was 0.205mg /1g of plant material **[44]**. In other study, the total phenolic and flavonoid content in *O. sanctum* methanol extract were 17.65 and 9.85%, respectively.

Variability of total flavonoids and phenolics content in *Z. officinale* according to the parts of plants used was reported by Ali et al. 2010 **[33]**. The results showed that the amounts of phenolic were 39.1; 13.5; 8.5mg GAE/g and flavonoids were 7.05; 4.21; 1.77mg QE/ g, for leaves, rhizome and steam, respectively. Total phenolic and flavonoid contents of the methanolic extracts in leaves and rhizomes of *Z. officinale* were determined **[45]**. The total phenolic content values were 33.0 and 10.22mg/1g of dry plant material, respectively in leaves and rhizomes. The amounts of flavonoids were 5.554 and 3.66 mg QE/1g of plant material, respectively for leaves and rhizomes. In other study, *Z. officinale* seed methanol and ethanol extracts the values of 462.9 ± 10.9 and 400.2 ± 10.1 mg GAE of phenols were respectively detected. The total flavonoid content expressed with pyrocatechol equivalent (PE) were 286.5 ± 3.5 and 268.2 ± 3.1 mg PE/g extract of methanol and ethanol, respectively **[46]**. The total phenolic and flavonoid content of *Z. officinale* rhizome were 39.49mg tanic/g and 55.10mg/g, respectively **[47]**.

According to Maizura et al. 2011 **[48]**, peeled *Z. officinale* rhizomes were extracted by using juice extractor without the additional of solvent. The amount of total phenolic obtained was 101.56 mg GAE/100 g extract. The amounts of phenolic of *Z. officinale* were 9.0 and 16.42 mg GAE/100g while flavonoids content were 1.68 and 2.95 µg QE/g of extract obtained respectively with ultrasonic and solvent extraction methods **[37]**.

Vaidya et al. 2014**[49]** reported a high amount of polyphenol of 175.51 mg GAE/1g and 325.28 mg GAE/1g for fresh and dried extracts of *R. officinalis*, respectively. However, he reported the lower amount of total flavonoids, 132.80µg QE/1g and 131.73µg QE/1g for fresh and dried extracts, respectively. The total phenolics content of *R. officinalis* leaves methanol extract was found to be 49.9 mg GAE/1g **[50]**. Shan et al. 2005 **[51]**, also found comparable value (50.7mg GAE/1g) of total phenolic content of *R. officinalis* leaves and stems methanol extracts. The total phenolics and flavonoids content of *R. officinalis* air part extract (80% MeOH-H_2O v/v) were 33.67mg GAE/1g and 13.25mg QE/1g, respectively **[52]**.

The total phenolic content of *R. officinalis* leaves water extract was found to be 185mg GAE/1g of extract **[53]**. In other study, the total phenolic and flavonoids content of *R. officinalis* leaves water extract were 13.44% and 9.54%, respectively, and in ethanolic (95%) extract total phenolic and flavonoids were 18.75% and 12.65%, respectively **[36]**. The total amount of phenolic and flavonoids present in the water extracts of *R. officinalis* was found to be 42.58 µgCE and 269.84 µg QE/g of extract, respectively **[54]**.

El-Moein et al. 2012**[55]** reported that *E. globules* contain the highest terpenoids content (10.2%), followed by phenolic compounds content (5.0%) while glycoside and flavonoids content have the lowest value (0.2 and 0.05 %), respectively. The total phenolic and flavonoids content of crude extract obtained by macerating 1g of *E. globules* bark with acetone-water (700:300, v/v) containing 0.5% acetic acid were 518.88mg GAE/g CE and 4.76 mg QE/g CE, respectively **[56]**. In 70% ethanolic extract of *E. globules* leaves, the total phenolic and flavonoids content were found to be 235.87mg GAE and 35.76mg RE/ 1g plant material, respectively **[57]**. Pereira et al. 2014 **[58]** reported lower amount of total phenolic content, 62.10 mg GAE/g dw plant material in 70% methanolic extract. According to Hassine et al. 2012 **[59]**, the total phenolics content in ethanolic extract was 143.4 mg GAE/g. By extracting 1 g of fine powdered of *E. globules* leaves with MeOH three times, the filtrate was evaporated till 2/3 part remained than 10 ml of extract was further diluted with DMSO and analyzing the total polyphenolic and flavonoids content of this extract the values of 167µg/ml and 185.0µg/ml, were obtained, respectively **[60]**. The reviewed total phenolic and flavonoid content of those plants are summarized in **Table 1.**

Table1. Total phenolic and flavonoids contents

Plant species	TP content (mg GAE/ 1g plant material)	TF content (mg QE/ 1g plant material)
Ocimum sanctum	3.65	0.205
Zingiber officinale	17 ±12.27	4.18±1.90
Rosemarinus officinalis	53.3±14.71	22.74±7.7
Eucalyptus globules	102.75±57.4	35.03±1.03

Fingerprinting and quantification of actives compounds

Imen et al. 2012 **[43]** described a qualitative and quantitative analysis of polyphenolic compounds of *O. basilicum* using a reverse-phase HPLC method. Mobile phase A (98% water and 2% acetic acid) and mobile phase B (68% water, 30% acetonitrile, and 2% acetic acid) was used for HPLC analysis. A linear gradient of 10 to 95%B was run for 90 min at a follow rate of 1 ml min^{-1} and detection was at 280 nm. The identity of the phenolic acids was confirmed by co-chromatography on HPLC with authentic standards, and quantification was performed using a standard curve in the range of 0.1 to 1µg of standards. The main compounds identified were rosmarinic, gentisic and caffeic acids while other minor compounds identified were gallic, coumaric, syringic, vanillic, p-OH-benzoic and ferulic acids.

The content of 1.3% and 0.5% for ursolic acid were found in methanolic and aqueous extracts of *O. sanctum*, respectively **[5]**. Leaves of *O. sanctum* contain 70% eugenol, carvacrol 3% and eugenol-methyl ether 20% **[7]**. Also, the amounts of borneol and vanillin (2.27%) were obtained in *O. sanctum* leaves essential oil **[14]**.

El-Bedawey et al. 2010 **[47]** developed a separation and identification of phenolic compounds of *Z. officinale* using HPLC and ODC-2 column and MeOH: Ammonium acetate (12: 88 v/v, pH= 5.4) as mobile phase. The amount of chlorogenic acid (102.49 mg/g) was the highest flowed by cinnamic acid (29.43 mg/g) and chrisin acid (4.09mg/g).

Gingerol [5-hydroxy-1-(4-hydroxy-3-methoxy phenyl) decan-3-one] together with shogaol and paradols were also identified as predominant pungent constituents of *Z. officinale* rhizome **[22]**.

Chemical analysis of *R. officinalis* extracts proven that this plant contain several compounds belong mainly to the classes of phenolic acids, flavonoids, diterpenoids and triterpenes **[36]**. According to El-Aziz et al. 2014 **[61]**, the amounts of cinnamic acid, vanailic acid and ferulic acid were 192.929, 152.607 and 76.876 mg/100g extract, respectively. Tsai et al. 2008 **[42]** developed method for identified and quantified phenolics compounds in methanolic extracts of *R. officinalis* by using a reverse-phase high-performance liquid chromatography (HPLC). The gradient elution programme was as follows: (solvent A; water/acetic acid 98:2 (v/v) and solvent B; methanol/ acetic acid 98:2 (v/v)): 15% B to 40% B, 30 min; 40% B to 75% B, 10 min; and 75% B to 85% B, 5 min. Detection was at 325 nm for caffeoyl derivatives, at 254 nm for rutin, myricetin, and quercetin, and at 263 nm for kaempferol. Myricetin (5.16mg), quercetin (2.81mg), chlorogenic acid (2.44mg), rutin (1.90mg), kaemferol (0.90mg) and caffeic acid (0.81mg) per 1g of methanol extract were quantified.

The subsequent fragmentation of negative and positive ions in the HPLC-ESI/MS/MS mode was used for identified and quantified of compounds in different plant extracts**[62, 63]**. Ultra performance liquid chromatography-tandem mass spectrometry (UPLC–MS/MS) was performed for phenolic characterization of *R. officinalis* extract **[63]**. Mobile phase was consisted with A (0.4% formic acid in water) and B(acetonitrile), the column and auto-sampler temperatures were held at 45 and 20^{o}C, respectively. The separation and elution gradient was set to last 42.0 min, changing from 2% B at 1min to 98% B at 30 min and to 2% B at 38 min, at a flow rate of 0.3 ml/min. Mass spectrometry was performed using TQ detector equipped with an electrospray ionization source (ESI) at positive ionization mode. Within 42 min of single experiment run, 23 different phenolic compounds were revealed. Among phenolic, caffeic acid was present in a good amount (9.87%) followed by p-hydroxy benzoic acid (8.12%) and rosmarinic acid (3.71%). Flavonoids such as 4'-methyl tectochrysin (18.63%), 4', 5, 7, 8-tetrahydroxyflavone (6.12%), genkawanin (3.78%) and cirsimaritin (3.07%) were dominant. Among phenolic diterpenes, epirosmanol (21.38%), methyl carnosate (10.19%), carnosic acid (6.12%) and carnosol (3.61%) were detected in significant amounts.

Stefan et al. 2015 **[57]** developed an HPLC-UV-MS method for simultaneous determination of polyphenols in *E. globules*. The identification and quantification of polyphenolic compounds was carried out using an Agilent Technologies 1100 HPLC Series system (Agilent, Santa Clara, CA, USA) equipped with G1322A degasser, G13311A binary gradient pump, column thermostat, G1313A autosampler and G1316A UV detector. The HPLC system was coupled with an Agilent 1100 mass spectrometer (LC/MSD Ion Trap SL). For the separation, a reverse-phase analytical column was employed (Zorbax SB-C18 100x 3.0 mm i.d., 3.5 µm particle) and the work temperature was set at 48°C. The detection of the compounds was performed on both UV and MS mode. The UV detector was set at 330 nm until 17.5 min, then at 370 nm. The MS system operated using an electrospray ion source in the negative mode. The mobile phase was a binary gradient: MeOH and acetic acid 0.1% (v/v). The elution started with a linear gradient, beginning with 5% MeOH and ending at 42% MeOH for 35 min; then 42% MeOH for the next 3 min. The chlorogenic acid was found at lower quantity (<0.02 µg/g) while flavonoid compounds such as hyperoside (666.4µg/g), quercitrin (287.8µg/g), rutin (48.6µg/g) and isoquercitrin (38.9µg/g) were quantified together with two flavonol compounds myricetin (92.3µg/g) and luteolin (34.4 µg/g).

Antioxidant activity

The second metabolites are often associated with various positive health effects associated on plant medicines including antioxidant effects, decreases in the risk of cardiovascular diseases, anti-cancer mechanisms, antimicrobial, antitussive and anti-inflammatory activities **[57, 64-67]**. The proximate linear correlation between antioxidant activity and phenolics content were reported in various plant species **[42, 48, 49]**. Anjali et al. 2013 **[68]** showed that total phenolic content had positive correlation with antioxidant capacity since the plant extracts rich in phenolics exhibited highest antioxidant and reducing activities of plant species. The measurements of antioxidant capacity of plant extracts showed a linear correlation between the antioxidant properties and the total phenolic and flavonoids content in *R. officinalis* extracts **[50, 52]**. As reported by Kim et al. 2011**[54]**, the high correlation coefficients were found between the total phenolic content and DPPH radical scavenging activity (r =0.9158) while the flavonoid content exhibited moderate correlation coefficients and DPPH radical scavenging activity and superoxide anion radical scavenging activity (respectively, r = 0.5430, r = 0.5598) for 13 plant species including *R. officinalis*.

Other researchers, however, have reported a poor linear correlation between antioxidant activity and phenolics content. As reported by Stefan et al. 2015 **[57]** the total phenolic and flavonoid content of *E. globules* were 235.87mg GAE/1g and 35.76mgQUE/g while *C. ficifolia* contained amount of 108.51mgGAE/g and 44.44mg QUE/1g, respectively. However, the author does not correlate the antioxidant activities with total phenolic content, since the obtained result suggested a link between the higher content of flavonoids in *C. ficifolia* and its high antioxidant activities. The same conclusion was also made for antibacterial activity against both Gram-positive and Gram-negative since *C. ficifolia* extract showed higher antibacterial activities than *E. globulus*.

Kasparavičienė et al 2013 **[34]** proven that antioxidant activity of substances may not be solely characterized by the total phenolic components and their particular structural characteristics. It has been reported that rosmarinic acid, linoleic acid, apigenin, cirsimaritin isothymusin, isothymonin and caryophyllene exhibited antioxidant, anti-inflammatory, antiviral and antibacterial activities **[10, 69-71]**. Oreintin and vicenin were also shown to provide protection against radiation-induced chromosomal damage in human blood lymphocytes **[10]**. Eugenol, linoleic acid and oleanic acid were reported to for anti- inflammatory and allergic properties of *O. sanctum* **[20, 72]**. The structure-antioxidant activity of 17-pentatricontene, N,N-diphenyllauramide and *O*-benzyl-N-tert-butoxycarbonyl-D-serine isolated from *E. globules* has been discussed **[55]**.

Antitussive and expectorant activities

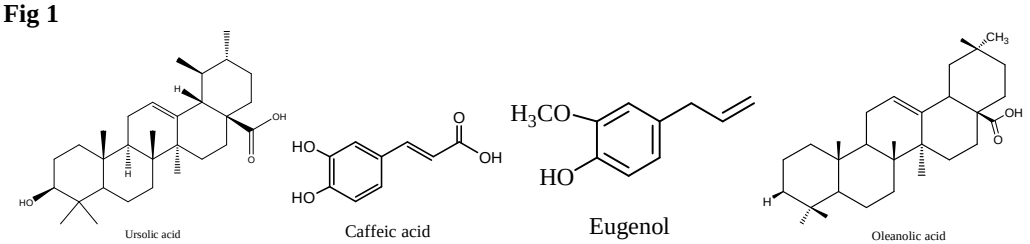
Various extracts and natural compounds with antitussive and expectorant activities have been reported **[73]**.The aqueous and methonolic extracts of *O. sanctum* showed antitussive activity on citric acid induced cough model in conscious guinea pigs**[5, 74]**. Aqueous extract at dose of 1.55 g per kg body wt. showed a reduction from 17.17 to 5.17 (72.5% inhibition) while methanolic extract at the dose of 0.875 g per kg body wt. showed a reduction from 15.5 to 9.83 (35.4% inhibition). In addition to other present active compounds in this plant, the authors suggested ursolic acid for responsible of this antitussive activity.

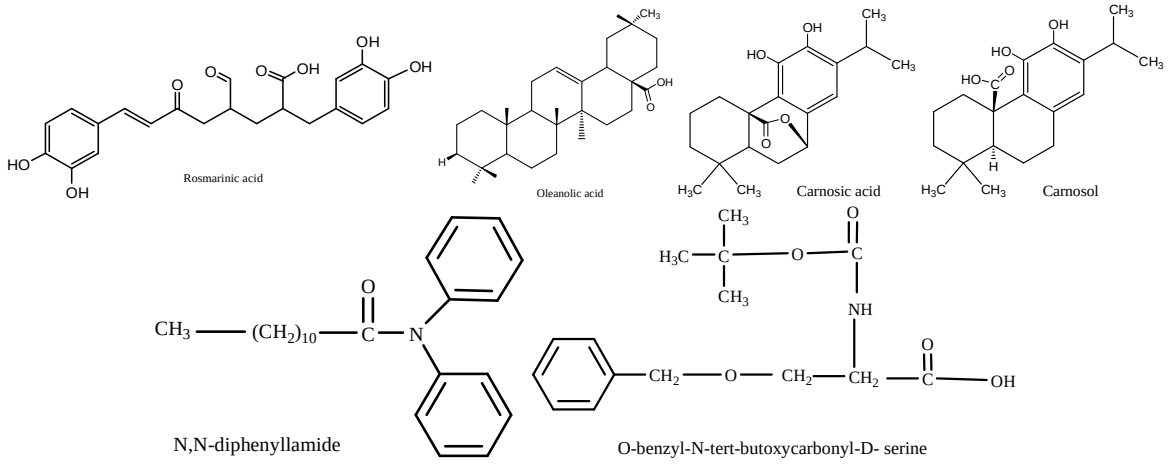
A polyherbal cough syrup containing *O. sanctum* produced 54%, 7%, 75% reduction in cough bouts at the dose level of 1, 2, 3 ml respectively after 1hr of drug administration on citric acid induced cough model in guinea pig. The antitussive activity of *O. sanctum* was attributed to eugenol, rosmarinic acid, carvacrol, methyl eugenol, camphene, α-cymene and ρ-cymene **[75]**.

Phenolic substances present in *Z. officinale*, generally, possess strong anti-inflammatory and antioxidative properties and exert substantial anticarcinogenic and antimutagenic activities **[76, 77]**. The antitussive activity of *Z. officinale* was attributed to zingiberene, camphene, ß-pinene, myrcene, limonene, 1,8-cineole , ß-phellandrene **[78]**. 6-Shogaol isolated from this plant was also reported for expectorant and antitussive activity**[79]**.

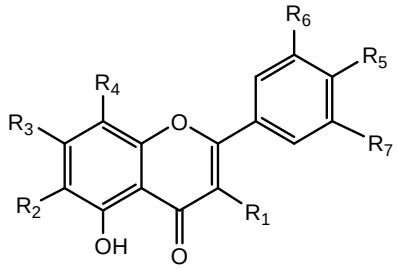
The expectorant activity of *E. globules* was attributed to crystallized resin, cymenes, terpenes, flavonoids including quercetin, tannins and volatile oils **[80, 81]**.

The antitussive and expectorant of fractions and compounds from plants have been shown to work as effectively as codeine in the throat, decreasing irritations and producing expectorant effects. One proposed explanation is that in the same way that carbenoxolone, is able to stimulate tracheal mucus secretion, it is also able to stimulate tracheal mucus secretions and hence produce demulcent and expectorant effects. The compounds are helpful remedy for coughs as they facilitate the movement of mucus from the respiratory tract **[82]**. However, in other study the antitussive mechanism action of *O. sanctum* was suggested by central nervous system probably mediated by both opioid system & GABA-ergic system **[5**]. The chemical structures of major antitussive and antioxidant activities compounds isolated from *Ocimum sanctum, Zingiber officinale*, *Rosamarinus officinalis* and *Eucalypus globules* are given at **Fig 1**





Chemical structure



Name of compound

1. Hyperoside: R_1=*O*- βGlc- αRha, R2=R4=R6=R7= H; R_3=R5=OH
2. Isoquercitrin: R_1=*O*-β-D-Glu; R_2=R_4=R_7=H; R_3=R_5=R_6=OH
3. Rutin: R_1=*O*-α-L-Rha-(1-6)-β-DGlu; R_2=R_4=R_7=H; R_3=R_5=R_6=OH
4. Myricetin: R1=R5=R6=R7=OH; R2=R3=H
5. Quercitrin: R_2=R_4=R_7=H; R_1=R_3=R_5=R_6=OH
6. Quercetin: R_2=R_3=R_4=R_7=H; R1=R_5=R_6=OH
7. Luteolin: R_1=R_2=R_4=R_7=H; R_3=R_5=R_6=OH
8. Apigenin: R_1=R_2=R_3=R_4=R_6=R_7=H, R_5=OH
9. Kampferal: R_1=R_2=R_5=R_6=R_7=H; R_3=OH; R_4=*O*-[(Z)-2-Methyl-2-butenol]

Figure 1: Chemical structure of major antitussive and antioxidant activities compounds

CONCLUSION

As shown in this paper, these plant species have highly complex chemical composition containing variety of biologically active compounds. Some of them have been well studied for their pharmacological actions and researchers are working to find new bioactive principles. By standardization of the herbal drug and development of modern dosage form for the herbal bioactive, we can achieve the global acceptance of traditional medicines. Further we will be able to quantify the active ingredients present in the crude drugs, as it affected by variety of factors either by natural like different climatic conditions or by poor manufacturing and storage conditions. The suitable extraction method for respective compounds could be chosen in order to have desired pharmacological effect. As shown in this report, UV-Vis spectrophotometric method is mostly used as simple, rapid, efficiency for routine estimation of various components in medicinal plants. Hence this current review will be a potent bio-prospecting tool for the discovery of new antitussive leads.

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