Preliminary phyto-chemical analysis and biological activity of *Hyptis suaveolens* (L.) (Lamiaceae)

Azhagu Raj R, M Gomathi, A Prakasham, Krishna Priya, V Narayananayar, P Mahesh and S Siva Subramanian

Abstract

Phytochemical screening and biological activity prediction provide imperative ideas for the development of new drug against deadly diseases. The medicinal plant *Hyptis suaveolens* extract was prepared by cold percolation method by using polar and non-polar solvents. The biological activity and pharmacologically active major compounds present in the medicinal plant *H. suaveolens* were studied by using cheminformatics tools. In this study, the phytochemical analysis results showed that thirteen (13) secondary metabolites of present in the methanolic extract, followed by aqueous extract (12) and acetone extract (11). The PASS predicted pharmacological activities of the compounds beta-caryophyllene, sabine, beta-pinene, and eucalyptol showed that they are Analgetic, Analgesic Antieczematic, Antipruritic, Antipruritic, Allergic, Antiprotozoal (Leishmania), and antifungal, antiparasitic and anti-inflammatory activities. This suggests that the medicinal plant *H. suaveolens* could be used to develop the novel drugs in near future for the betterment of mankind.

Keywords: Medicinal plant, Phytochemistry, Cheminformatics, PASS and biological activity

1. Introduction

*Hyptis suaveolans* (L.) Poit. Commonly known Wilayati Tulsi (Tamil) is an aromatic, bushy shrub or woody herb. It is normally found in profoundly disturbed soils, and is called 'ruderal' species (Wulff, 1987) [11]; Kamaraj et al., 2016 [21]. *Hyptis suaveolens* Poit (Lamiaceae), is an aromatic scented herb (Gavani and Paarakh, 2008) [3]. The plant is used as a stimulant, carminative for wounds, infection of the uterus, antimicrobial, anti-inflammatory, anticarcinogenic, anticancer, parasitic, skin diseases and antioxidant in the management of free radical mediated diseases (Gavani and Paarakh, 2008) [13]. Leaf juice and paste administered to relieve soreness for insect bite and snake bite, juice is used as a lotion and also leaf extract is taken thrice daily and tender leaves is used to treat pneumonia in children (Ganesan et al., 2006) [4].

Phytochemical analysis provided that significant ideas for the development of new drugs against deadly diseases. In recent times, a number of studies have been reported on the phytochemistry of the medicinal plants across the world (Edeoga, et al., 2005 [5]; Aliyu, et al., 2008 [6]; Ayoola, et al., 2008 [7]; Johnson, et al., 2008 [8]; Maridass, et al., 2008a [9]; Maridass, et al., 2008b [10]; Majaw and Moirangthem, 2009 [11]; Devmurari and Jivani, 2010 [12]; Ujowundu, et al., 2010 [13]; Rafa Rasool, et al., 2010 [14]; Usha and Bopai, 2011 [15], Hamad, et al., 2011 [16] and Azhaguraj et al., 2016) [17]. Phytochemical analysis of medicinal plants can help the manufacturers for identification and selection of raw materials for drug production.

Prediction of Activity Spectra for Substances (PASS) is a software product designed as a tool for evaluating the general biological potential of an organic drug-like molecule (Filimonov, et al., 1995 [18]; Poroikov et al., 1996) [19]. PASS provides simultaneous predictions of many types of biological activity based effects exclusively on the structural organic compounds. Thus the program PASS can be used to estimate the biological activity profiles for virtual molecules, prior to their chemical synthesis and biological testing (Filimonov, et al., 1995 [20]; Poroikov et al., 1996 [19]; Filimonov and Poroikov, 1996 [20]; Poroikov, 2001 [21]).

PASS inlet predicates biological activity spectrum on the basis of the structural formula of the compound. Establishing the quantitative relationship between molecular structure and broad biological effects which have been long-standing a challenge in science (Poroikov et al., 1996 [19] and Poroikov, 2001 [21]). Pa and Pi are the estimates of probability for the compound to be active and inactive respectively for each type of activity from the biological activity spectrum, their values vary from 0.000 to 1.000 (Poroikov et al., 1996 [19]; Filimonov and
Poroikov, 1996) [20]. PASS has been used to predict the Antimalarial activity (John de Britto, 2008) [22]. Anti-HIV activity (Maridass et al., 2008), Activities of plant secondary metabolites (Maridass 2008) [10]. Activities of essential oils (Abiya Chelliah, 2008) [23], Antitumor activity (Azhaguraj et al., 2010) [24] and Phenazine derivatives (Azhaguraj et al., 2012) [25]. In this background, the present study intended to evaluate the qualitative phytochemical analysis and biological activity of the H. suaveolens secondary metabolites.

2.0 Materials and Methods

2.1. Collection and Identification

*Hyptis suaveolens* was collected from the regions of Thirumayam, Pudukottai District, Tamil Nadu, India during the period of October 2015 to April 2016. The plant was shade dried for two weeks continuously. The shade-dried leaf was partially powdered using the domestic blender (Preethi Pvt. Ltd) and stored in airtight container for further experiments.

2.2. Preparation of the Crude Extracts

From these stocks, secondary metabolites of the medicinal plant *H. suaveolens* (50 gram) was extracted sequentially using (200 mL) non-polar and polar solvents such as hexane, petroleum ether, acetone, chloroform, methanol (Merk, Mumbai) and double distilled water. The samples were kept in dark for 96 hours, after incubation, the extracts thus obtained were decanted and filtered. The clear extracts were consequently concentrated using rotary vacuum evaporator and kept in dark bottles in 4° C until further use (Johnson et al., 2012) [26].

2.3. Phytochemical Analysis

The various qualitative chemical tests were performed for establishing the profile of the aromatic plant *H. suaveolens* extracts for its chemical composition. Qualitative phytochemical analyses were done by using the procedures of Kokate (1994) [27] and Kokate et al., (1995) [28] Sofowara (1993) [29], Tease and Evans (1989) [30], Harborne (1973) [31], Brindha, (1991) [32], Edeoga et al., (2005) [5], Savithramma et al. (2011) [33] and Azhaguraj et al. (2015) [34].

2.3.1. Alkaloids

Test solution one mL (crude extract) shaken with 2N HCL (01mL). Aqueous layer formed, decanted and to which 1 or 2 drops of Mayer’s reagent is added, White turbidity or precipitate indicates the presence of alkaloids (Harborne, 1973) [31].

2.3.2. Steroids

One mL of the extract was treated with 0.5 mL of acetic anhydride and cooled in ice. This was mixed with 0.5 mL of chloroform and 1 mL of concentrated sulphuric acid was then added carefully by means of a pipette. At the separations level of the two liquids, a reddish-brown ring was formed, as indicators of the presence of steroids (Harborne, 1973) [31].

2.3.3. Reducing sugars

Two mL of extracts is added to Fehling A (2 mL) and Fehling B (2 mL) solution, in a test tube, heated in water bath. The appearance of brick Red precipitate indicates the presence of reducing sugars (Harborne, 1973) [31].

2.3.4. Tannins

500 milligram of the dried powdered samples was boiled in 20 mL of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black coloration indicates the presence of tannins (Harborne, 1973) [31].

2.3.5. Phlobatannins

Deposition of a red precipitate when an aqueous extract of each plant sample was boiled with 1% aqueous hydrochloric acid was taken as evidence for the presence of Phlobatannins (Edeoga et al., 2005) [5].

2.3.6. Saponins

About 2 gram of the powdered sample was boiled in 20 mL of distilled water in a water bath and filtered. 10ml of the filtrate was mixed with 5 mL of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil or Coconut and shaken vigorously, then observed for the formation of emulsion. (Kapoor, et al., 1969 [35], Smolenski et al., 1974 [36] and Majaw and Moirangthem, 2009) [11].

2.3.7. Coumarins

Two mL of extracts is added to few drops of 10% of sodium hydroxide and chloroform were mixed in a test tube. Formation of yellow colour indicates the presence of Coumerin (Brindha et al., 1991) [32].

2.3.8. Flavonoids

Two mL of extracts treated with methanol or ethanol and Para dimethyl amine benzaldehyde, few drops of Con. HCL. Appearance of Red or Pink colour indicates the presence of flavonoids (Edeoga et al., 2005) [5].

2.3.9. Terpenoids

Add Five mL of each extract was mixed in 2 mL of chloroform, and concentrated H2SO4 (3 ml) was carefully added to form a layer. A reddish brown colouration of the inter face was formed to show positive results for the presence of terpenoids (Edeoga et al., 2005) [5].

2.3.10. Triterpenoids

Add Two mL of extracts, a piece of Tin chloride, and three drops of Thionyl chloride and a appearance of a Violet or purple colour indicates the presence of triterpenoids (Harborne, 1973) [31].

2.3.11. Cardiac glycosides:

The Five mL of each extracts was treated with 2 mL of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1 mL of concentrated sulphuric acid. A brown ring of the interface indicates a deoxy sugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer (Edeoga et al., 2005) [5].

2.3.12. Glycosides

0.5 gram of crude powder was dissolved in 5 mL of methanol. 10 ml of 50% HCl was added to 2 mL of methanolic extract in a test tube. The mixture was heated in a boiling water bath for 30 min. 5 ml of Fehling’s solution was added and the mixture was boiled for 5 min to observe a brick red precipitate as an indication for the presence of glycosides (Tease and Evans, 1996) [30].
2.3.13. Anthraquinones
Only Five mL of the extract solution was hydrolysed with diluted Conc. H2SO4 extracted with benzene. 1 mL of dilute ammonia was added to it. Rose pink coloration suggested the positive response for anthraquinones (Trease and Evans, 1996) [30].

2.3.14. Phenolic groups
Take Two mL alcoholic of test extracts and added one drop of FeCl3, in a test tube. The appearance of intense color indicates the presence of phenolic groups. (Harborne, 1973) [31].

2.3.15. Quinones
Take Two mL of extracts is added to 1-2 drops of 10% sodium hydroxide in a test tube. The Appearance of Blue green or red colour indicates the presence of Quinone (Brindha et al., 1991) [32].

2.3.16. Amino acids
Add two mL of extracts is added to 1-2 drops of 1% Ninhydrin and few drops of alcohol in a test tube. The appearance of Blue or violet colour indicates the presence of amino acids.

2.3.17. Essential Oils
Add Two 2 mL of extracts is added to few drops of alcoholic K2Cr2O7 and added 1-2 drops of phenolphthalein. The Soap Formation of solution indicates the presence of essential oils.

2.3.18. Aromatic acids
Add Two mL of extracts is added to 0.2mg of NaHCO3 in a test tube. The appearance of brisk effervescence indicates the presence of aromatic acids.

2.3.19. Xanthoproteins
Add Two mL of extracts is added to few drops con.HNO3 and added three mL NH3. The appearance of Reddish orange precipitate indicates the presence of Xanthoprotein.

2.3.20. Carbohydrates
Take Two mL of extracts is added to few drops 20% NaoH solution. The Solution turned brown on heating indicates the presence of carbohydrates.

2.3.21. Anthocyanins
Add two mL of aqueous extract is added to 2 mL of 2N HCL and ammonia. The appearance of pink-red turns blue-violet indicates the presence of anthocyanins, (Paris, 1969) [33].

2.3.22. Leucoanthocyanins
Five mL of aqueous extract added to 5 ml of isomyl alcohol. Upper layer appears red in colour indicates for presence of leucoanthocyanins (Paris, 1969; Savithramma et al., 2011) [37] [33].

2.3.23. Emodins
Two mL of NH OH and the 3mL of Benzene were added to the extracts. Appearance of red colour indicates the presence of emodins (Rizk, 1982; Savithramma et al., 2011) [38] [33].

2.3.24. Gum and Mucilage
The extract (100mg) was dissolved in 10 ml of distilled water and to this, 25 ml of absolute alcohol was added with constant stirring. White or cloudy precipitate indicated the presence of gums and mucilage’s.

2.4. PASS
The chemical structures of the aromatic plant Hyptis suaveolens major metabolite beta-caryophyllene, sabinene, beta-pinene, and eucalyptol were obtained from the Pubchem compound (NCBI) repository (http://www.ncbi.nlm.nih.gov/pubchemcompound). The chemical structures were drawn using the Chem Sketch package 11.0 belonging to the ACD Chem Laboratory. The biological and pharmacological activity of H. suaveolens metabolites were predicated by PASS tools (Poroikov et al., 1996; Filimonov and Poroikov, 1996 and Poroikov, 2001) [19] [20] [21].

3.0. Results and Discussion
Phytochemical screening of twenty different chemical compounds such as steroids, alkaloids, phenolic groups, saponins, tannins, flavonoids, anthraquinones, reducing sugars, triterpenoids, terpenoids, cardiac glycosides, glycosides, phlobatamins, quinones, aromatic acids, essential oils, anthocyanins, leucoanthocyanins, emodins, gum and mucilage, carbohydrates, coumarins, aminoacids and xanthoprotein) were tested in the medicinal plant H. suaveolens leaf crude extracts. Thus out of (6×20 =120) tests for the presence or absence of the above compounds (Table 1).

The preliminary phytochemical analysis were performed by using various polar and non-polar solvent such as hexane, petroleum ether, acetone, chloroform, methanol and aqueous extracts of the medicinal plant Hyptis suaveolens. The phytochemical results showed that various kind of phyto constituents such as carbohydrates, cardiac glycosides, coumerin, essential oils, flavonoids, phenolic groups, quinones, reducing sugars, saponins, steroids, tannins, terpenoids, xanthoprotein and phlobatamin were present in the Hyptis suaveolens leaf extract. Whereas alkaloids, aminoacids, aromatic acids, anthraquinones, anthocyanides, glycosides and triterpenoids were absent in all the solvent extracts of H. suaveolens (Table1).

Table 1: Phytochemical analysis of the medicinal plant Hyptis suaveolens leaf extract

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>Hexane</th>
<th>Petroleum Ether</th>
<th>Acetone</th>
<th>Chloroform</th>
<th>Methanol</th>
<th>Aqueous</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td></td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Amino Acids</td>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Anthocyanines</td>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Anthraquinones</td>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Aromatic Acids</td>
<td></td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td></td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Coumerins</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Essential oils  
Flavonoids  
Glycosides  
Phenolic groups  
Pholobatanins  
Quinones  
Reducing sugars  
Saponins  
Steroids  
Tannins  
Terpenoids  
Triterpenoids  
Xanthoproteins

(-) indicates absence (+) indicates presence

**Table 2:** Pharmacological activity profile of *H. suaveolens* - Beta-Caryophyllene

<table>
<thead>
<tr>
<th>Pharmacological activities</th>
<th>Probability of active (Pa)</th>
<th>Probability of inactive (Pi)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antineoplastic</td>
<td>0.915</td>
<td>0.005</td>
</tr>
<tr>
<td>Antieczematic</td>
<td>0.897</td>
<td>0.005</td>
</tr>
<tr>
<td>Apoptosis agonist</td>
<td>0.847</td>
<td>0.005</td>
</tr>
<tr>
<td>Antiinflammatory</td>
<td>0.745</td>
<td>0.011</td>
</tr>
<tr>
<td>Antipsoriatic</td>
<td>0.734</td>
<td>0.005</td>
</tr>
<tr>
<td>Dermatologic</td>
<td>0.734</td>
<td>0.006</td>
</tr>
<tr>
<td>Antileukemic</td>
<td>0.638</td>
<td>0.007</td>
</tr>
<tr>
<td>Antifungal</td>
<td>0.582</td>
<td>0.020</td>
</tr>
<tr>
<td>Antipruritic, allergic</td>
<td>0.488</td>
<td>0.045</td>
</tr>
<tr>
<td>Antiprotozoal (Leishmania)</td>
<td>0.470</td>
<td>0.029</td>
</tr>
</tbody>
</table>

**Table 3:** Pharmacological activity profile of *H. suaveolens* - Eucalyptol C 10 H18 O (PubChem CID: 2758)

<table>
<thead>
<tr>
<th>Pharmacological activities</th>
<th>Probability of active (Pa)</th>
<th>Probability of inactive (Pi)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respiratory analeptic</td>
<td>0.910</td>
<td>0.004</td>
</tr>
<tr>
<td>Analgetic</td>
<td>0.840</td>
<td>0.004</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td>0.825</td>
<td>0.004</td>
</tr>
<tr>
<td>Phobic disorders treatment</td>
<td>0.833</td>
<td>0.022</td>
</tr>
<tr>
<td>Antiinfective</td>
<td>0.807</td>
<td>0.005</td>
</tr>
<tr>
<td>Analgesic</td>
<td>0.768</td>
<td>0.005</td>
</tr>
</tbody>
</table>

*H. suaveolens* major secondary metabolite beta-Caryophyllene biological activity profile showed that the a variety of pharmacological activity such as, Antineoplastic, Antieczematic Apoptosis agonist, Antiinflammatory Antipsoriatic, Dermatologic, Antileukemic, Antifungal, Antipruritic, allergic and Antiprotozoal (Leishmania) etc were predicated through the chem-informatics tool PASS (Table.2).
Table 4: Pharmacological activity profile of H. suaveolens Sabinine C_{16} H_{16} (PubMed CID: 10887971)

<table>
<thead>
<tr>
<th>Pharmacological activities</th>
<th>Probability of active (Pa)</th>
<th>Probability of inactive (Pi)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antieczematic</td>
<td>0.947</td>
<td>0.003</td>
</tr>
<tr>
<td>Antineoplastic</td>
<td>0.891</td>
<td>0.005</td>
</tr>
<tr>
<td>Antinflammatory</td>
<td>0.853</td>
<td>0.005</td>
</tr>
<tr>
<td>Antipsoriatic</td>
<td>0.800</td>
<td>0.004</td>
</tr>
<tr>
<td>Immunosuppressant</td>
<td>0.582</td>
<td>0.031</td>
</tr>
<tr>
<td>Prostate disorders treatment</td>
<td>0.588</td>
<td>0.014</td>
</tr>
<tr>
<td>Antipruritic, allergic</td>
<td>0.555</td>
<td>0.021</td>
</tr>
<tr>
<td>Antipruritic</td>
<td>0.495</td>
<td>0.036</td>
</tr>
<tr>
<td>Antiprotozoal (Leishmania)</td>
<td>0.398</td>
<td>0.049</td>
</tr>
<tr>
<td>Nitric oxide scavenger</td>
<td>0.312</td>
<td>0.005</td>
</tr>
</tbody>
</table>

Table 5: Pharmacological activity profile of H. suaveolens beta-Pinene C_{10} H_{16} (PubMed CID: 440967)

<table>
<thead>
<tr>
<th>Pharmacology activities</th>
<th>Pa</th>
<th>Pi</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antieczematic</td>
<td>0.902</td>
<td>0.005</td>
</tr>
<tr>
<td>Dermatologic</td>
<td>0.709</td>
<td>0.007</td>
</tr>
<tr>
<td>Antineurotic</td>
<td>0.664</td>
<td>0.051</td>
</tr>
<tr>
<td>Antineoplastic</td>
<td>0.648</td>
<td>0.035</td>
</tr>
<tr>
<td>Analgetic</td>
<td>0.624</td>
<td>0.017</td>
</tr>
<tr>
<td>Antioseptorotic</td>
<td>0.589</td>
<td>0.009</td>
</tr>
<tr>
<td>Bone diseases treatment</td>
<td>0.588</td>
<td>0.009</td>
</tr>
<tr>
<td>Antiflammatory</td>
<td>0.601</td>
<td>0.032</td>
</tr>
<tr>
<td>Antipruritic</td>
<td>0.541</td>
<td>0.026</td>
</tr>
<tr>
<td>Antipruritic, allergic</td>
<td>0.521</td>
<td>0.032</td>
</tr>
<tr>
<td>Immunosuppressant</td>
<td>0.511</td>
<td>0.040</td>
</tr>
</tbody>
</table>

PASS was used to predict the biological activity profile of secondary metabolites like Taxol, Vinblastine, Vincristine Topotecan, Irinotecan, Etoposide and Teniposide. Secondary metabolites are also well known for their effectiveness on living species (Jeeva et al. 2006[43]). Biological activity of major flavanoids from a medicinal herb, Boesenbergia pandurata Holtt (Zingiberaceae) was predicted through PASS (Maridass, et al., 2008)[44].

Biological activity for compounds present in five major spics namely, cinnamon Cinnamom umverum, nutmeg Myristica fragrans, garcencia Garcinia cambogia, all spices Pimenta dioica and black pepper Piper nigrum, for their biological activity as promising therapeutic compounds (Riju et al., 2009[45]). Rajendra Prasad et al. (2011) [46] studied the mechanism of action, pharmacological activity and toxic and side effects of 1,3,5-Trisubstituted-2-Pyrazoline derivatives.

The phytochemical results showed that various kind of phyto constituents such as carbohydrates, cardiac glycosides, coumerin, essential oils, flavonoids, phenolic groups, quinones, reducing sugars, saponins, steroids, tannins, terpenoids, xanthoprotein and pholobatanins were present in the Hypitis suaveolens leaf extract.

4.0. Conclusion
The present study concludes that the medicinal plant Hypitis suaveolens presence of various kind of phyto-constituents such as cardiac glycosides, coumerin, essential oils, flavonoids, phenolic groups, quinones, reducing sugars, saponins, steroids, tannins, terpenoids, xanthoprotein and pholobatanins were present in the leaf extracts. They could possess several pharmacological activity such as, Analactic, Analgesic Antieczematic, Antipruritic, Antipruritic, allergic, Antiprotozoal (Leishmania), antifungal, antiparasitic and antiinflammatory activities etc. PASS tool was useful for the study of biological activity of medicinal plant Hypitis suaveolens. From this current studies, it can be concluded that PASS predictions of biological activity spectrum gives a fair approach for the corresponding reported activities of Hypitis suaveolens and determining the other valuable insights of other side effects.

Conflict of Interests
The authors do not have any conflict of interests.

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