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Azhagu Raj R
Department of Zoology, St. Xavier's College, Palayamkottai, Tamil Nadu, India

M Gomathi
Department of Physics, Thiruvalluvar Government Arts College, Rasipuram, Tamil Nadu, India

A Prakasam
Department of Physics, Thiruvalluvar Government Arts College, Rasipuram, Tamil Nadu, India

Krishna Priya
Department of Botany, J.J. College of Arts and Science, Pudukkottai, Tamil Nadu, India,

V Narayanayyar
Department of Botany, J J College of Arts and Science, Pudukkottai, Tamil Nadu, India.

P Mahesh
Department of Zoology, Annamalai University, Chidambaram, Tamil Nadu, India

S Siva Subramanian
Department of Botany, J J College of Arts and Science, Pudukkottai, Tamil Nadu, India.

Correspondence
S Siva Subramanian
Department of Botany, J J College of Arts and Science, Pudukkottai, Tamil Nadu, India.

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

Azhagu Raj R, M Gomathi, A Prakasam, Krishna Priya, V Narayanayyar, 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 chem-informatics 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, sabinene, beta-pinene, and eucalyptol showed that they are Analeptic, 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 [1]; Kamaraj *et al*., 2016) [2]. *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) [3]. 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]; Rafia Rasool, *et al*., 2010 [14]; Usha and Bopaiah, 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, Pudukkottai 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 (Merck, 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 4º 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], Trease 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 acid 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 indicates 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 was 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 benzaldyhyde, 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 H2S04 (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 triterpneoids (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 deoxysugar 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 (Trease and Evans, 1996) [30].

2.3.13. Anthraquinones
Only Five mL of the extract solution was hydrolysed with diluted Conc. H_2SO_4 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 $FeCl_3$, in a test tube. The appearance of intense colour 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 $K_2Cr_2O_7$ 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 $NaHCO_3$ 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.HNO_3 and added three mL NH_3. 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 of 10% 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[37]).

2.3.22. Leucoanthocyanins
Five mL of aqueous extract added to 5 ml of isoamyl 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, phlobatannins, 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 gylcosides, coumerin, essential oils, flavonoids, phenolic groups, quinones, reducing sugars, saponins, steroids, tannins, terpenoids, xanthoprotein and pholobatanins 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

Phytochemicals	Hexane	Petroleum Ether	Acetone	Chloroform	Methanol	Aqueous
Alkaloids	-	+	-	-	-	-
Amino Acids	-	-	-	-	+	+
Anthocyanides	-	-	-	-	-	-
Anthraquinones	-	-	-	-	-	-
Aromatic Acids	-	-	+	-	+	-
Carbohydrates	-	-	+	-	+	+
Cardiac glycosides	-	+	+	-	+	+
Coumerins	+	+	+	+	+	-

Essential oils	-	+	+	-	-	+
Flavanoides	+	+	+	+	+	-
Glycosides	-	-	-	-	-	-
Phenolic groups	-	-	+	+	+	+
Pholobatanins	+	+	+	+	+	+
Quinones	-	-	-	-	-	+
Reducing sugars	_	_	+	++	_	+
Saponins	+	+	+	+	+	+
Steroids	-	-	-	-	++	-
Tannins	+	+	+	+	+	+
Terpenoids	-	-	+	-	+	+
Triterpenoids	-	-	-	-	-	-
Xanthoproteins	-	+	-	+	+	+

(-) indicates absence (+) indicates presence

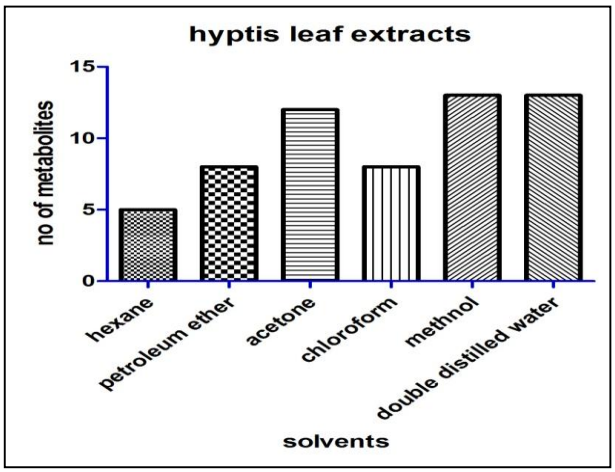

Fig 1: Total number of phytochemicals present in the medicinal plant *Hyptis suaveolens* leaf extracts

Hyptis suaveolens (Laminaceae), is an aromatic scented herb (Gavani and Paarakh, 2008) [3]. The plant is used as stimulant, carminative for wounds, infection of uterus, antimicrobial, anti-inflammatory, anticarcinogenic, anticancer, parasitic, skin diseases and antioxidant in the management of free radical mediated diseases (Gavani and Paarakh, 2008) [3]. Tender leaves is used to treat pneumonia in children. Leaf juice and paste (Ganesan *et al.*, 2006) [4] administered to relieve pain for insect bite and during snake bite juice is used as lotion and also leaf extract is taken thrice daily (Reddy *et al*. 2010) [39]. *Hyptis* was used to treat fever and root extract is taken repeatedly for urinary complications. Young twigs and leaves are useful against skin diseases (Ajit Kumar Das *et al*., 2008) [40]. Leaf paste is applied on sores, skin infections and fungal infection (Prashant Kumar and Vidyasagar 2008) [41].

The presence of flavonoids and tannins in the plants were phenolic compounds. Plant phenolics are a major group of compounds that acts as primary antioxidant are potent water soluble and free radical scavengers, which prevent oxidative cell damage, have strong anticancer activity (Gavani and Paarakh, 2008) [3]; Mbatchou, *et al*. (2010) [42]. Saponin has the property of binding with cholesterol, bitterness and haemolytic activity in aqueous solution Mbatchou, *et al*. (2010) [42].Tannins could also meant that it is an astringent, with wound healing and anti-parasitic properties. *H. suaveolens* saponins, which is used to stop bleeding and in treating wounds and ulcers as it is helps in red blood cell coagulation (Reddy *et al*. 2010) [39].

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

Pharmacological activities	Probability of active (Pa)	Probability of inactive (Pi)
Antineoplastic	0,915	0,005
Antieczematic	0,897	0,005
Apoptosis agonist	0,847	0,005
Antiinflammatory	0,745	0,011
Antipsoriatic	0,734	0,005
Dermatologic	0,734	0,006
Antileukemic	0,638	0,007
Antifungal	0,582	0,020
Antipruritic, allergic	0,488	0,045
Antiprotozoal (Leishmania)	0,470	0,029

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 3: Pharmacological activity profile of H. *suaveolens*- Eucalyptol $C_{10}H_{18}O$ (PubChem CID: 2758)

Pharmacological activities	Probability of active (Pa)	Probability of inactive (Pi)
Respiratory analeptic	0,910	0,004
Analeptic	0,840	0,004
Rheumatoid arthritis treatment	0,825	0,004
Phobic disorders treatment	0,833	0,022
Antiinfective	0,807	0,005
Analgesic	0,768	0,005

Hepatic disorders treatment	0,763	0,004
Antineoplastic (colorectal cancer)	0,721	0,005
Antiparasitic	0,693	0,006
Antiprotozoal	0,723	0,004

Table 4: Pharmacological activity profile of H. *suaveolens* Sabinene C_{10} H_{16} (PubChem CID: 10887971)

Pharmacological activities	Probability of active (Pa)	Probability of inactive (Pi)
Antieczematic	0,947	0,003
Antineoplastic	0,891	0,005
Antiinflammatory	0,853	0,005
Antipsoriatic	0,800	0,004
Immunosuppressant	0,582	0,031
Prostate disorders treatment	0,588	0,014
Antipruritic, allergic	0,555	0,021
Antipruritic	0,495	0,036
Antiprotozoal (Leishmania)	0,398	0,049
Nitric oxide scavenger	0,312	0,005

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

Pharmacology activities	Pa	Pi
Antieczematic	0,902	0,005
Dermatologic	0,709	0,007
Antineurotic	0,664	0,051
Antineoplastic	0,648	0,035
Analeptic	0,624	0,017
Antiosteoporotic	0,589	0,009
Bone diseases treatment	0,588	0,009
Antiinflammatory	0,601	0,032
Antipruritic	0,541	0,026
Antipruritic, allergic	0,521	0,032
Immunosuppressant	0,511	0,040

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 spices namely, cinnamon *Cinnamom umverum*, nutmeg *Myristica fragrans*, garcinia *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 gylcosides, coumerin, essential oils, flavonoids, phenolic groups, quinones, reducing sugars, saponins, steroids, tannins, terpenoids, xanthoprotein and pholobatanins were present in the *Hyptis suaveolens* leaf extract

4.0. Conclusion

The present study concludes that the medicinal plant *Hyptis suaveolens presence of* various kind of phyto-constituents such as cardiac gylcosides, 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, Analeptic, 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 *H. 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 *H. 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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