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RESEARCH ARTICLE

Qualitative and quantitative analysis of various phytoconstituents in H*yptis suaveolens L. i*n different solvents

Nishi Yadav and Renu Mishra

Abstract: Plants represent the reservoir of secondary metabolites, responsible for its medicinal and aromatic properties. This study is aimed to analyze leaf extract of *Hyptis suaveolens* qualitatively and quantitatively in terms of their phytoconstituents. The extracts were screened for the presence of various phytoconstituents using preliminary chemical tests for qualitative analysis. For quantitative analysis, total phenolic content was determined by Folin-Ciocalteu method, total flavonoid content by aluminum chloride assay and total alkaloid content was determined by spectrophotometric assay based on bromo cresol green reaction. The analysis revealed the presence of bioactive compounds such as alkaloids, glycosides, proteins, saponins, flavonoids, tannins and phenols in aqueous fraction. The ethanolic fraction contained glycoside, alkaloid, flavonoid, tannin, phenol, steroid and fats. In the methanolic fraction only alkaloid, steroid, saponin and proteins were present, whereas acetone extract contained glycoside, steroid, fats and resins. Maximum phytoconstituents were observed in the aqueous and ethanolic extract of the plant. In quantitative analysis among the extracts tested, ethanol extract of *H. suaveolens* leaves showed maximum amount of flavonoid content (2.98±0.52 mg/100mg), phenolic content (2.28±0.41 mg/100mg) and alkaloid content (0.77±0.33 mg/100mg) as compared to aqueous extract. In methanol extract only, alkaloid content (0.21±0.12 mg/100mg) was determined. Bioactive compounds contained in the leaf extracts included flavonoids, tannins, phenols, alkaloids and glycosides which suggests that constituents of the plant extracts could serve as a source of drugs useful in the treatment of various diseases.

Key words: Atropine, gallic acid, *Hyptis suaveolens,* phytochemicals, quercetin

Introduction

Plants have been utilized in conventional medication for a few thousand years. From the time immemorial, human developments have been investigating and utilizing different plants and plant products to treat dangerous diseases. Various plants species and their uses as prescription are significantly outstanding to indigenous communities in different parts of the world. The intelligence about the utilization of traditional herbal medicines steadily perishes, while few traditional tribal social sections believed in the utilisation of natural drugs, and are still exercising the ability of herbal healing effectively (Shah *et al* 2015).

Phytochemicals are the concoctions secreted by different parts (bark, leaves, flower, roots and seeds) of a plant. Plants are an excellent resource of an extensive range of compounds, for example, phenols, terpenoids, nitrogen containing mixes, vitamins, and secondary metabolites. These bioactive constituents of plants have different actions, for example, antibacterial, antifungal, haemolytic, antioxidant, antimicrobial, anti-inflammatory, antitumor, antimutagenic, anticarcinogenic and diuretic activities (Khare *et al* 2011).

The present study is carried out with a plant, *Hyptis suaveolens* Linn. commonly known as wilayti tulsi. This plant belongs to family Lamiaceae. The plant is easily available in roadsides and unused lands. This dicot plant, a resident to tropical America, is a yearly herb. Various parts of the plant have been utilized by customary healers in the treatment of different sicknesses and illness conditions. In the northern piece of Nigeria, an extract of the leaves is utilized for treating boils, skin inflammation and diabetes mellitus (Danmalam *et al*. 2009). The plant has been accounted for to have antifertility, anti-

✉ Nishi Yadav
nishiyadavsis18@gmail.com

Sri Sathya Sai College for Women, Bhopal (M.P.)

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inflammatory and antiplasmodial properties (Chukwujekwu *et al.,* 2005; Grassi *et al.,* 2006). It is a significant source of basic oils, alkaloids, flavonoids, phenolics, saponins, terpenoids and sterols. Silver and Bostian (1993) have recorded the utilization of natural foodstuffs as novel antibacterial drugs. There is a critical need to recognize novel substances dynamic towards extremely resistant pathogens (Recio, 1989; Cragg *et al*., 1997). The main objective of this study to analyze qualitative and quantitative phytoconstituents in leaf extract of *Hyptis suaveolens*.

Materials and methods

Collection of plant material

Twigs of *Hyptis suaveolens* Linn. were collected from Bhopal (M. P.). The plants were identified from Govt. M.V.M. College with the help of the project report of Dr. Madhuri Modak, who confirmed the identification from Botanical survey of India, Allahabad. The fresh leaves were separated from the plants. The separated leaves were washed and shade dried at room temperature. The leaves were then ground to a powder with the help of mechanical grinder and stored separately at room temperature in close containers for further studies. The voucher specimen is preserved in the Department of Botany, Sri Sathya Sai College for Women, Bhopal (Satish *et al* 2007).

Extraction of powdered plant material

Extraction method involves the separation of medicinally active portions of plant leaves by selective solvents through standard methods. Primarily the extracts may be used as medicinal agents in the form of tinctures and fluid extracts because primary extracts include mixture of bio-constituents like alkaloids, glycosides, phenols, terpenoids, flavonoids, alkaloids and glycosides but still further process is necessary (Azwanida 2015). The powder of *Hyptis suaveolens* leaves were subjected to extraction by hot soxhlet method.

Preparation of leaf extracts by Soxhlet method

50 gm of the dried powdered sample was extracted with 300 ml of solvents such as water, methanol, ethanol and acetone in a Soxhlet apparatus. The powdered plant material was filled in the thimble with the appropriate amount of solvent in the flask for extraction process. At the boiling point of the respective solvent, the temperature was maintained.

Qualitative analysis of selected plants

Phytochemical tests were carried out to test various bioactive compounds like alkaloids, saponins, flavonoids, terpenoids etc. in the leaf extracts of plant (Yadav and Agarwala 2011, Harborne 1995).

Quantitative analysis of bioactive compounds

Quantitative assessment is significant for determining the amount of phytoconstituents present in the plant. For this TPC, TFC and TAC in the leaf extract of *Hyptis suaveolens* Linn. plant was determined by standard procedure.

Estimation of total phenolic content (TPC)

Modified Folin-Ciocalteu method was used to estimate the total phenolic content of leaf extract of plant (Ainsworth and Gillespie, 2007). Gallic acid was utilised as standard for evaluation.

Estimation of total flavonoids content (TFC)

The TFC of leaf extract was determined by using the aluminium chloride method (Oueslati *et al.,* 2012). Quercitin was used as the standard for assessment.

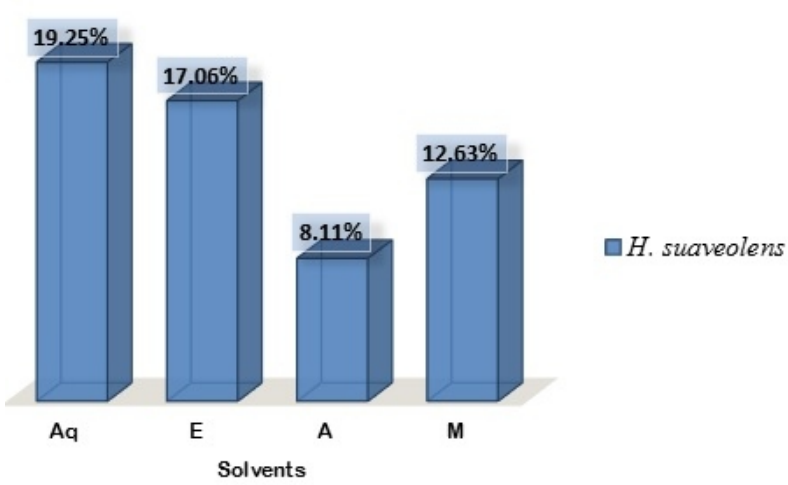
Estimation of total alkaloids content (TAC)

The complete alkaloid content in the sample was determined using a spectrophotometric technique based on the alkaloid-bromocresol green (BCG) reaction (Shamsa *et al.,* 2008).

Results

Preparation of leaf extract and calculation of yield

Preparation of leaf extract of *Hyptis suaveolens* was done by using Soxhlet apparatus. The percentage



Aq- Aqueous E- Ethanol A- Acetone M- Methanol

Figure 1: Percentage yield of plant extract from various solvents

Metabolites	Chemical Constituents	Chemical test	Solvents			
			Aq	E	A	M
Primary Metabolites	Carbohydrate	Molisch's test	-	-	-	-
		Fehling's test	-	-	-	-
	Proteins	Millon's test	+	-	-	+
		Xanthoprotiec test	+	-	-	+
	Fats and oil	Stain test	-	+	+	-
Secondary Metabolites	Alkaloids	Mayer's test	+	-	-	+
		Wagner's test	+	+	-	+
		Hager's test	-	+	-	-
	Glycosides	Borntrager's test	+	+	+	-
		Keller Killiani test	+	+	+	-
	Steroids	Salkowski test	-	+	+	+
		Liebermann Burchard test	-	+	+	+
	Saponins	Froth test	+	-	-	+
		Foam test	+	-	-	+
	Flavonoids	Alkaline reagent test	+	+	-	-
		Lead acetate test	+	+	-	-
	Tannins	Gelatin test	+	+	-	-
	Phenolics	Ferric chloride test	+	+	-	-
	Terpenoids	Copper acetate test	-	-	-	-
	Resins	Acetone water test	-	-	+	-

Table 1: Phytochemical screening of extracts of *Hyptis suaveolens*

yield of extract was obtained using the formula: W_2-$W_1/W_0 \times 100$, where W_2 is the weight of the extract and the container, W_1 the weight of the container alone and W_0 the weight of the initial dried sample (Anokwuru, 2011). Percentage yield was found to be 19.25% in aqueous, 17.06% in ethanol, 8.11% in acetone and 12.63% in methanol (Fig-1). Highest yield from extraction was obtained in aqueous fraction.

Qualitative Phytochemical analysis of leaves of *Hyptis suaveolens* plant extracts

The preliminary phytochemical screening that was carried out on the four different solvents from the leaves of *H. suaveolens* revealed the presence of various bioactive secondary metabolites. The leaf extracts 1mg per ml in different solvents such as acetone, methanol, ethanol and aqueous were screened for the presence of various bioactive compounds. The analysis revealed presence of bioactive compounds such as alkaloids, glycosides, phenols, steroids, flavonoids, tannins and saponins in different solvent fractions.

The results from the Phytochemical analysis of leaf extracts showed the presence of

Alkaloids, Glycosides, Proteins, Saponins, Flavonoids, Tannins and Phenols in Aqueous extract. Similar phytochemicals were present in ethanolic extract of the plant except Proteins and Saponins. Methanolic extract contained only Glycosides, Steroids, Saponins and Proteins. Acetone extract of plant gives positive results for glycosides, steroids, fats and resins. Overall the result showed that maximum phytoconstituents were present in aqueous followed by ethanol, methanol and acetone extract of plant leaves. Phytochemical characteristics of leaf extract of the plant in four different solvents tested are summarized in the Table 1.

Quantitative estimation of Plant *Hyptis suaveolens*

Based on the results obtained from preliminary phytochemical screening, the plant extract has shown positive response for the presence of major phytoconstituents. Hence, it was further subjected to quantitative estimation of phenols, flavonoids and alkaloids.

Total phenolic content estimation (TPC)

The total phenolic content for extracts of *Hyptis suaveolens* was estimated by Folin- Ciocalteu's method. Gallic acid was used as standard. The total phenolic content (TPC) was measured and expressed in terms of mg/100mg of gallic acid equivalent of dry extract sample. It was measured by using the equation obtained from the linear calibration curve: $Y = 0.011X+0.011$, $R^2= 0.998$, where X is the gallic acid equivalent (GAE) and Y is the absorbance. The phenolic content was estimated to be 2.280±0.410 mg GAE/100 mg for ethanol and 1.018±0.500 mg GAE/100 mg for aqueous (Table 2). The maximum phenol content was found in the ethanolic extract of *Hyptis suaveolens*. In methanolic fraction, phenols were not present.

Total flavonoids content estimation (TFC)

The total flavonoid concentration in plant extracts of leaves of *Hyptis suaveolens* was determined using the aluminium chloride method. It was measured and expressed in terms of mg quercetin equivalent/100 mg of dry extract. The standard equation was obtained from the linear calibration curve: $Y= 0.040X + 0.009$, $R^2=0.999$, where X is the quercetin equivalent (QE) and Y is the absorbance.

The quantity of flavonoids measured in the plant extracts is shown in (Table 2). It was found to be 2.981±0.520 mg QE/100 mg in ethanol extract and 1.272±0.460 mg QE/100 mg in the aqueous extract of *Hyptis suaveolens.* Flavonoid was found to be absent in methanolic fraction.

Total alkaloid content estimation (TAC)

The quantification of alkaloid in the leaf extract of *Hyptis suaveolens* was determined through spectrophotometric method which is based on the reaction of alkaloid and bromocresol green (BCG). In this method, atropine was used as standard. Total alkaloid content was calculated and expressed as atropine equivalent mg AE/100mg. The standard equation obtained from the calibration curve was: $Y=0.007X$, $R^2=0.999$, where X is the atropine equivalent (AE) and Y is the absorbance.
Total alkaloid content was estimated to be in the range of 0.414±0.420 mg AE/ 100 mg for aqueous extract, 0.771±0.330 mg AE/ 100 mg for ethanol and 0.218±0.12 mg AE/ 100 mg for methanol extract (Table-2).

The results revealed that the ethanolic extract of *Hyptis suaveolens* leaves have comparatively higher amount of TFC, TPC and TAC as compared to the aqueous extract. From the present investigation it was also estimated that the plant contained the highest flavonoid content followed by phenol content whereas alkaloid content was estimated to be in less quantity (Table-2).

Discussion

Phytochemical analysis of leaf extract of *H. suaveolens*

To explore the importance of any medicinal plant, the initial step is to screen for its phytochemicals, as it gives a broad idea regarding the nature of compounds present in it. Phytochemical screening aids as an initial step for future determination of its activity like antioxidant, anticancer, anti-inflammatory, antimutagenic, etc. Different phytochemicals possess various protective and therapeutic effects which are essential to prevent

diseases and maintain a state of well-being. The medicinal value of plants depends on the chemical substances that have a definite physiological action on the human body. The most important bioactive constituents of plants are alkaloids, tannins, saponins, terpenoids, steroids, glycosides, flavonoids and phenolic compounds (Hill 1952). In the present investigation, qualitative analysis of four different solvent extracts (aqueous, ethanol, acetone and methanol) of *H. suaveolens* leaves were analysed for presence of phytoconstituents (Table-1). The use of four solvents for extraction has revealed that the phytochemical composition of the extract varies with the solvent used.

Phytochemical screening of the plant revealed the presence of alkaloid, glycosides, phenols, saponins, tannins, proteins and flavonoids in the aqueous leaf extract. A recent work on the ethanolic leaf extract of *H. suaveolens* has also reported the presence of these components (Shaikat *et al.* 2012, Agarwal and Varma 2013). Earlier studies on phytochemical analysis of stem, leaf and root extract of *H. suaveolens* also showed the presence of these bioactive compounds. Their findings revealed that maximum constituents were present in the leaf extract of the plant (Pachkore *et al.* 2011).
In the present study methanol fraction contain only few phytoconstituents like alkaloids, steroids, proteins and saponins, although Kumar and Thampi (Kumar and Thampi 2015), reported maximum phytochemicals in the methanolic extract of plant. Several studies have reported the presence of bioactive compounds which are responsible for the medicinal properties of the plant that is used for treatment of various ailments (Naz *et al.* 2007).

TPC, TFC and TAC of *H. suaveolens* leaves

The results of this study indicate that the leaves of the plant contain flavonoids, tannins, phenols, steroids, saponins, carbohydrate and glycosides. These phytochemicals are known to be of therapeutic importance since they have biological roles. For example, flavonoids are shown to have antibacterial activity (Cushnie and Lamb 2005). Phenols have antioxidant activity (Chakraborthy *et al* 2012). Tannins are also reported to have antiviral, antitumour, wound healing and antiparasitic effects (Daniel *et al* 2011, Amokaha *et al* 2002). Hence, a quantitative evaluation of the flavonoids, total phenols and alkaloids was attempted to study the concentration of these phytochemicals. Similar studies were done by other researchers (Sharma *et al.* 2017). The total phenolic content for extract of *Hyptis suaveolens* was determined using the Folin - Ciocalteu's method. Total flavonoid content was estimated by using aluminium chloride method, whereas quantification of alkaloid was based on the reactions of alkaloid and bromocresol green.

Tannins and phenolic compounds are the major secondary metabolites responsible for the antioxidant activity. This activity is believed to be mainly due to their oxidation/reduction properties, which play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides (Long *et al.* 2006). In the present investigation, the concentration of phenols in the ethanolic and aqueous leaf extract of *H. suaveolens* was 2.280 and 1.018 mg/100mg, respectively. Total phenolic and flavonoid contents of *H. suaveolens* in methanolic extract were studied by Ghaffari *et al* 2014.

In the present study total flavonoid content was estimated to be in the range of 1.2 to 3.0 which was found to be similar to Sharma *et al* 2017. The plant is considered as being an important medicinal plant due to the presence of various phytochemicals (Shenoy *et al* 2009). Studies done by Koche *et al* 2010 revealed that the crude extracts of *H. suaveolens* contained the highest percentage of alkaloids and flavonoids (14.30 ± 0.36% and 12.60 ± 0.48%), respectively.

It has been reported that the aromatic plants act as a source of natural antimicrobial agents due to the presence of phenols and flavonoids (Lopez-Mejia *et al* 2014, Witaicenis *et al.* 2014). Quantification of TFC, TPC and TAC was estimated from other plants of family Lamiaceae by other workers (Geethika and Kumar 2007, Benabdallah *et al* 2016). Our studies revealed that total flavonoid content was found to be maximum as compared to phenol and alkaloid content. Alkaloid content was found to be lowest in quantification. Our findings disagree with the work of other author who reported maximum alkaloid content in the plant (Edeoga *et al.* 2006).

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