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HISTOCHEMICAL AND ANTIMICROBIAL ACTIVITY OF LAMIACEAE PLANTS (ANISOMELES INDICA O. KZE, HYPTIS SUAVOLENS POIT, LEONOTIS NEPETAEFOLIA R. BR., AND LEUCAS ASPERA SPR)

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ABSTRACT

Four Lamiaceae family plants were selected (*Anisomeles indica* O. Kze, *Hyptis suavolens* Poit, *Leonotis nepetaefolia* R. Br., and *Leucas aspera* Spr.) and secondary metabolites like alkaloids, ascorbic acids, tannins, polyphenols and terepenoids were identified using histochemical methods. Stem hand sections were treated with respective reagents and the colour indication proved the presence of respective compounds. Alkaloid showed higher concentration, tannins showed moderate concentration, while terpenoid showed lower concentration. Antimicrobial screening was conducted using ethanolic leaf extracts of these plants. Two ml of leaf extracts were introduced into 10 ml of Nutrient Agar Medium. Clinical strains of bacteria such as *Bacillus subtilis, Klebsiella pneumoniae, Proteus vulgaris* and *Streptococcus pneumoniae* were inoculated using streak plate method. *L. nepetaefolia* was most effective against the four microbes than the other leaf extracts.

KEYWORDS: Antibacterial activity, Histochemical methods, Secondary metabolites.

INTRODUCTION

Lamiaceae family is one of the largest and most distinctive family of flowering plants is represented by 45 genera with 574 species distributed worldwide (Erik and Tarikahya, 2004). Plants of this family are herbs or shrubs often with an aromatic smell. They are common in the Asia and other Mediterranean countries. Lamiaceae family is well represented; many members of this family are used as tea, spice or for medicinal purpose to treat cough, dysmenorrhea, fever, headache, heart diseases, stomachaches and wound healing (Nurdan Sarac and Aysel Vgur, 2007).

Lamiaceae plants synthesis many secondary metabolites, which help the plants to protect themselves against the aggression from other organisms. These secondary metabolites such as alkaloids, ascorbic acid, polyphenol, tannins or terpenoid are also used as bioactive substances in drug industry as natural therapies (Hammer *et al*., 1999). These secondary metabolites also processes antimicrobial activities inhibit microbial growth and Lamiaceae plant species are important because these plants are considered to have better antimicrobial activities and are locally available (Fabricant and Famsworth, 2001). Histochemical tests were useful to localize the secondary metabolites present in plants *in situ* condition. With this background, an attempt was made to study Histochemical and Antibacterial activity of four Lamiaceae plants viz. *Anisomeles indica* O. Kze., *Hyptis suavolens* Poit, *Leonotis nepetaefolia* R. Br. and *Leucas aspera* Spr.

MATERIALS AND METHODS

Plant Collection

Fresh plants of *A. indica, H. suavolens, L. nepetaefolia* and *L. aspera* were collected from the green-house maintained at Bishop Heber College, Tiruchirappalli, Tamilnadu, India and the stem anatomical sections were taken and histochemical tests were conducted to locate the bioactive compounds Shanmugam *et al.* (2010) and antibacterial activity the leaf extracts were carried over as per Shanmugam *et al.* (2010) methodology.

Histochemical Test

Alkaloid

Sections were stained in Dragendroff's reagent, it is prepared by the following formulation:

Bismuth nitrate + Glacial acetic acid: 20 ml + 8 ml water + 50% Potassium iodide.

Terpenoids

Sections were stained in 2,4-Dinitro phenyl hydrazin (DPPH).

Ascorbic acid

Sections were treated in 10% silver nitrate in 3% acetic acid in 4 to 24 hr in dark, then washed with distilled water than stained in 1% crystal violet in ethanol.

Polyphenols
Sections were treated in equal volume of 10% sodium nitrate + 10-20% urea + 10% acetic acid for 3-4 minutes then 2 volume of 2N Sodium Hydroxide was added.

Tannins
Section were treated in 10% Formalin solution containing 2% Ferric sulphate or ferric chloride.

Determination of antibacterial activity
Microbial strains used to evaluate the antimicrobial activity were two Gram-positive bacterial strains *Streptococcus pneumoniae* (NCBT 054) and *Bacillus subtilis* (NCBT 012), two Gram-negative bacterial strains *Proteus vulgaris* (NCBT 038) and *Klebsiella pneumoniae* (NCBT 022). Microorganism were obtained from Microbial Culture Collection Centre, National College Biotechnology (NCBT) Lab, Tiruchirappalli, Tamil Nadu, India.

Preparation of leaf extract
The leaf material of respective plants were washed thoroughly with distilled water to remove any possible impurities. It was air dried to remove the moisture completely, one gram of leaf material was chopped into small pieces and ground well with 10 ml ethanol, and then filtered using membrane filter Minisart (Sartorius Stedinm, Biotech.) (0.20 µm). The extract was used to determine antibacterial activity against clinical strains of bacteria. The crude alcoholic extracts were tested for the antibacterial effect using different concentration. Petridishes containing 10 ml of Nutrient Agar and 2 ml of respective leaf extract per plate. Positive control as Nutrient agar and the negative control streptomycin 20 µl were used. The plates were incubated at 37°C ± 1°C for 48 hr. The antibacterial activity was evaluated by the streaking method. All tests were repeated three times.

RESULTS AND DISCUSSION

Histochemical Analysis
Histochemical tests of *A. indica* and *L. aspera* showed a positive reaction to alkaloids, polyphenols, ascorbic acids, tannins and terpenoids in the free hand section of stem whereas the other two plants such as *H. suaveolens* and *L. nepetaefolia* showed positive reaction to secondary metabolite like alkaloids, ascorbics acids, polyphenols, tannins while negative reaction for terpenoids (Table 1).

In *A. indica*, high amount of alkaloids were located from the cuticles towards the xylem parenchyma tissue and less amount in the pith region. Cortex, sclerenchyma, phloem, showed black silver colour or red brown colour, indicates the presence of ascorbic acids. The cherry red is seen across the epidermis cortex sclerenchyma localized the presence of polyphenols. The presence of tannins is localized by the least distribution of blue green in the cortex of *A. indica*. The orange colour is expressed in the sclerenchymatous tissue which identified the presence of terpenoids.

In *H. suaveolens*, the epidermis, xylem, pith regions showed golden yellow colour, which indicates the high amounts of alkaloids. Epidermis and xylem parenchyma expressed red brown colour to prove the presence of ascorbic acids. The epidermal region having the cherry red colour showed the presence of polyphenols and light blue green colour distributed overall the tissue indicates the presence of tannins. *H. suaveolens* showed absence of terpenoids.

In *L. nepetaefolia*, part of schlerenchyma region and phloem showed the presence of alkaloids with golden yellow colour. The black silver indicates the presence of ascorbic acids distributed only in the pith region. Cherry red colour distributed in the phloem and xylem parenchyma indicates the presence of polyphenolic compounds. Blue green coloured distributed schlerenchyma of *L. nepetaefolia* indicates the present of tannin compound.

L. aspera stem showed moderate amount of alkaloids, this is identified by golden yellow colour distributed in epidermis and pith. The xylem parenchyma and outer layer of pith region have identified the presence of ascorbic acids with black silver and red brown colour. Cuticle, phloem and xylem sowed cherry red colour is due to the presence of polyphenols compounds. The region of collenchyma and the xylem expressed the blue green colour indicates the presence of tannin.

Alkaloids and distributed naturally in plants, they are classified as organic amine, piperidine, indolizidine, quinolizidine, izidine, pyrrolizidine, indole, tropane and many other alkaloids (Aniszewski, 2007). The distribution of alkaloid in plant show large differences in the location and also plants to plants such variations are found in the present study also as reported by Dai *et al*. (2007) and Lin *et al*. (2007). The presence of ascorbic acids, polyphenols, tannins and terpenoids in plants were studied by Butler *et al*. (2000), Carvalho (2007), Rinaldo *et al.* (2010) and Gallon *et al.* (2015) and according to them the histochemical study to locate these chemical compounds differ in their location in plant system, the present study also in accordance with their study.

Antibacterial activity
The antimicrobial activity was conducted by using ethanolic leaf extract as positive control, streptomycin (20 µl) is used as a negative control. The antimicrobial activity of ethanolic extracts of *L. nepetaefolia* on four different human pathogenic organisms have showed maximum effects against Gram-negative bacteria such as *Klebsiella pneumoniae* and *Proteus vulgaris* followed by Gram-negative bacteria *Streptococcus pneumoniae* and *Bacillus subtilis*. The antimicrobial activity of ethanolic extract indicated that among the four medicinal plants used *L. nepetaefolia* have shown better impact on all the four pathogenic bacteria and strains when compared to rest of the plants.

The results of this study suggest the possibility of using these four Lamiaceae plants as potential sources of bioactive compounds with antibacterial activity as suggested by Ceren Yavuz *et al.* (2017). Aromatic plants have great importance in pharmaceutical industries in relation to have the potential as antimicrobial agent the present results are in accordance with the work of Nurdan Sarac and Aysel Ugur (2007), Revathi *et al.* (2011) and Sh. Fahimi *et al.* (2015).

Table 1: Histochemical Test.

Compound	Reagent used	Colour	Observation in different plants			
			Anisomeles indica	***Hyptis suavolens***	***Leonotis nepetaefolia***	***Leucas aspera***
Alkaloid	Dragendroffs Reagents	The plant tissue appears golden yellow	+	+	+	+
Polyphenols	Two volume of 2N Sodium Hydroxide	The plants tissue appear cherry red colour	+	+	+	+
Ascorbic acids	1% crystal violet	The plants tissue appear black silver or red brown	+	+	+	+
Tannins	Ferric sulphate or ferric chloride test	The plants tissue appear Blue or Bluegreen	+	+	+	+
Terpenoids	2,4-Dinitrophenylhydrazin (DPPH)	The plants tissue appear orange colour	–	–	+	–

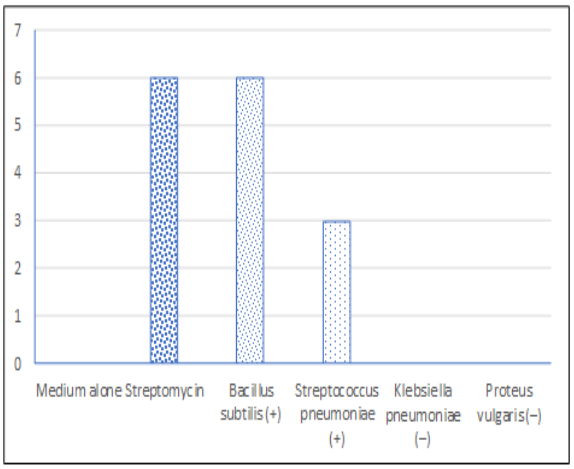

Fig. 1: Antibacterial activity of *A. indica.*

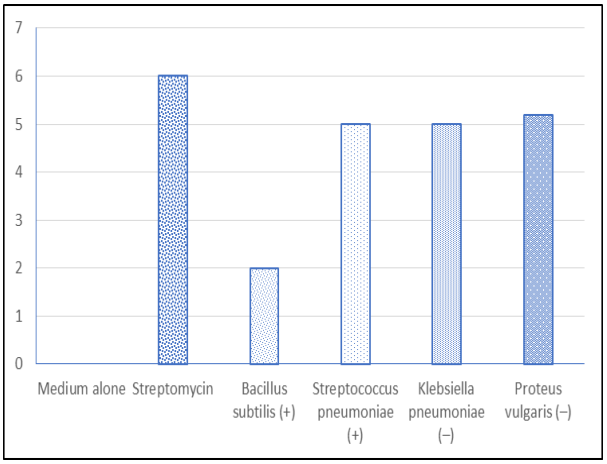

Fig. 3: Antibacterial activity of *L. nepetaefolia.*

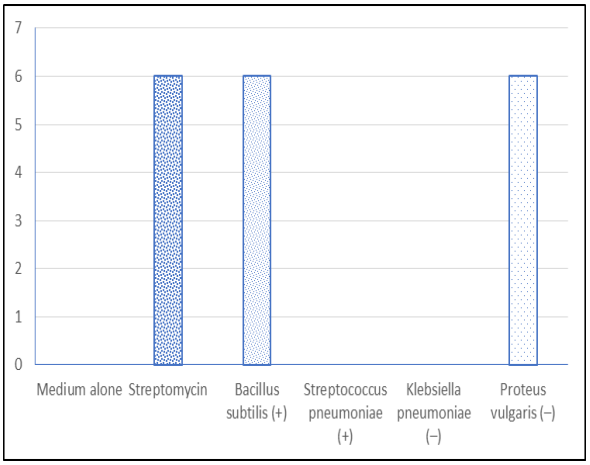

Fig. 2: Antibacterial activity of *H. suaveolens.*

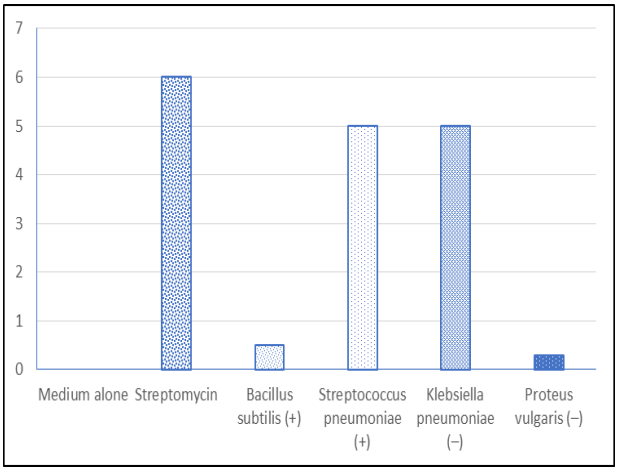

Fig. 4: Antibacterial activity of *L.aspera.*

CONCLUSION

The histochemical studies are necessary for identification of secondary metabolite of plants species. The variations found in the chemical constituent of the plants studied, viz. alkaloids, ascorbic acids, polyphenols, tannins and terpenoids are due to their metabolic activities. The potent antibacterial activity of these plants attributed to the various phytochemical constituents present in the extract. The purified compounds will serve as antibacterial phytochemical drug in pharmaceutical industries.

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