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HISTOCHEMICAL AND ANTIMICROBIAL ACTIVITY OF PLANTS WITH ANOMALOUS SECONDARY STRUCTURES

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ABSTRACT

Four anomalous secondary structure plants were selected [*Achyranthes aspera* L. (Amaranthaceae), *Bignonia suberosa* Roxb. (Bignoniaceae), *Boerhaavia diffusa* L. (Nyctaginaceae) and *Nyctanthes arbor-tristis* (Oleaceae)] and secondary metabolites like alkaloids, ascorbic acids, polyphenols tannins, 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 and ascorbic acid were present in all the four plants in higher concentration. *Nyctanthes arbor-tristis* have shown the presence of Terpenoid and Tannin also in higher concentration. Tannin is present in moderate concentration in *A. aspera, B. suberosa and B. diffusa*. Terpenoid present in lower concentration in *A. aspera, B. suberosa* and *B. diffusa.* Polyphenol is present only in *A. aspera* and found to be absent in other three plants. 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. *A. aspera* as well as *B. diffusa* leaf extract was effective for *K. pneumoniae* and *S. pneumoniae* as total inhibition of growth in the culture plants. *B. suberosa* ineffective to all the four bacteria, whereas *N. arbor-tristis* is effective for *P. vulgaris* bacteria as total inhibition of growth was noticed.

KEYWORDS: Anomalous secondary structure, Antibacterial activity, Histochemical methods, Secondary metabolites.

INTRODUCTION

Angiosperm plants of some family members show anamolous secondary growth, it is an increase in plant girth due to the abnormal activities of cambium or cork cambium or associated with the formation of multiple cambia in cortex, medullary region or in the pith region, the anomalous secondary growth is very specific to some plants belong to the families Amaranthaceae, Aristolochiaceae, Asclepiadaceae, Bignoniaceae, Chenopodiaceae, Loganiaceae, Oleaceae, Onagraceae, Nyctaginaceae, Salvadoracea and some other families. These plants also show medicinal importance, their leaf extract have shown many bioactive compounds, therefore a study was conducted to locate the bioactive compound by Histochemical studies and also to evaluate their potentials as antibacterial activities (Nurdan Sarac and Aysel Vgur, 2007; Sathya and Phawa, 2018; Mostafa and Bakis, 2018).

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 plant species are important because 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 plants with anomalous secondary structures. Many plants posses stellar structure of the normal type, but some of them have unusual structure and these structure differ from the normal type and these are called anomalous structure and the secondary structure is called anomalous secondary growth. The plants selected for this work namely *A. aspera* cambia is present in separate strips and produced many pith bundles as anomalous structure. *B. suberosa* is characterized by the presence of phloem in wedges of xylem, *B. diffusa* possess several cambia arise successively in a centrifugal direction and resulted in the formation of many concentric rings of vascular bundles

found in medullary region called medullary vascular bundles. In *N. arbor-tristis*, the vascular bundles are seen below the epidermis and the bundles are inversely oriented found at the four corners of the stem (Esau, 1965; Fahn, 1967; Cutter, 1969, 1971).

MATERIALS AND METHODS

Plant Collection

Fresh plants of *A. aspera, B. suberosa, B. diffusa* and *N. arbor-tristis* 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 as per Shanmugam *et al.* (2010) and Vasant *et al.* (2016) and antibacterial activity the leaf extracts were carried over as per Pandyal *et al.* (2017) 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.

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.

Terpenoids

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

Determination of antibacterial activity

Microbial strains used to evaluate the antimicrobial activity were two Gram-positive bacterial strains *Bacillus subtilis* (NCBT 012) and *Streptococcus pneumoniae* (NCBT 054), two Gram-negative bacterial strains *Klebsiella pneumoniae* (NCBT 022) and *Proteus vulgaris* (NCBT 038). 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 alkaloid and ascorbic acid showed a positive reaction to alkaloids and ascorbic acids in higher concentration in the free hand section of stem of all the four plants. Polyphenols not to be found in *B. suberosa, B. diffusa* and *N. arbor-tristis* but found in less concentration in *A. aspera*. Tannins were found in moderate concentrations in *A. aspera, B. suberosa* and *B. diffusa*, whereas in *N. arbor-tristis* found in higher concentrations. Terpenoids are found in less concentration in the case of *A. aspera* and *B. diffusa*, but in higher concentrations in *N. arbor-tristis* (Table-1).

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

In *B. suberosa*, 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 dark blackish brown colour distributed overall the tissue indicates the presence of tannins.

In *B. diffusa*, part of schlerenchyma region and ground tissue showed the presence of alkaloids with golden yellow colour. The black silver indicates the presence of ascorbic acids distributed in the vascular bundle region. Cherry red colour distributed in the phloem and xylem parenchyma indicates the presence of polyphenolic compounds.

N. arbor-tristis stem showed good amount of alkaloids, this is identified by golden yellow colour distributed in epidermis, hypodermis, secondary xylem regions. The xylem parenchyma and outer layer of pith region have

identified the presence of ascorbic acids with black silver colour. Cuticle, phloem and xylem sowed cherry red colour is due to the presence of polyphenols compounds.

Alkaloids 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 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), Gallon *et al.* (2015) and Sathya and Phawa (2018) 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 which have shown total inhibition at this concentration. The antimicrobial activity of ethanolic leaf extracts of *A. aspera, B. suberosa, B. diffusa* and *N. arbor-tristis* plants on four human pathogenic organisms have showed different effects against gram-positive and Gram-negative bacteria. *A. aspera* extract is much effective for *S. pneumoniae* and *K. pneumoniae* (75% growth inhibition), *B. subrata* is moderately effective for *B. subtilis* and *P. vulgaris* (25% growth inhibition), *B. suberosa* extract is moderately effective (50% growth inhibition) for *B. subtilis* and less effective (25% growth inhibition), for *K. pneumoniae*, *P. vulgaris* and *S. pneumoniae*, *B. diffusa* extract is very effective for *S. pneumoniae* and *K. pneumoniae* (75% growth inhibition) whereas *N. arbor-tristis* extract is moderately effective for *S. pneumoniae* but very effective for *P. vulgaris* (75% growth inhibition) (Table-2).

The results of this study suggest the possibility of using *A. aspera* as well as *B. diffusa* leaf extract as potential antibacterial activity for *S. pneumoniae* and *K. pneumoniae*. These two 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), Sh. Fahimi *et al.* (2015), Mostafa and Bakri (2018) and Manadhar *et al*. (2019).

Table 1: Histochemical Test.

Compound	**Reagent used**	**Colour**	**Observation in different plants**			
			Achyranthes aspera	***Bignonia suberosa***	***Boerhaavia diffusa***	***Nyctanthes arbor-tristis***
Alkaloid	Dragendroffs Reagents	The plant tissue appears golden yellow	+++	+++	+++	+++
Ascorbic acids	1% crystal violet	The plants tissue appear black silver or red brown	+++	+++	+++	+++
Polyphenols	Two volume of 2N Sodium Hydroxide	The plants tissue appear cherry red colour	+	–	–	–
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	+	–	+	+++

+++ : Higher concentration
++ : Moderate concentration
+ : Less concentration
– : Not found

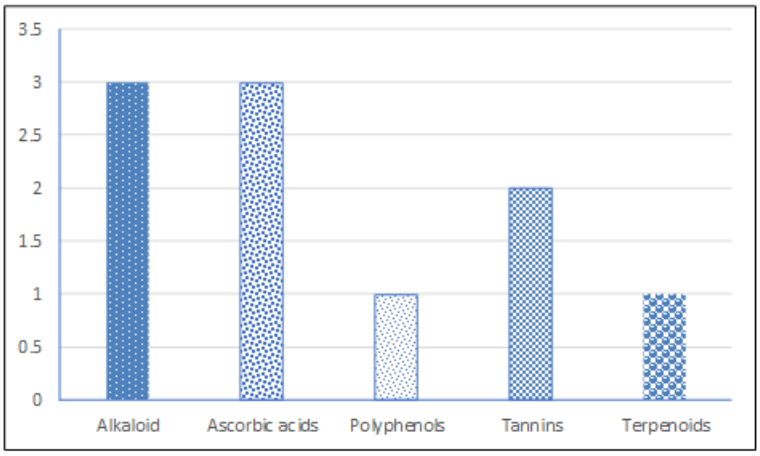

Fig. 1: Histochemical test of *A. aspera.*

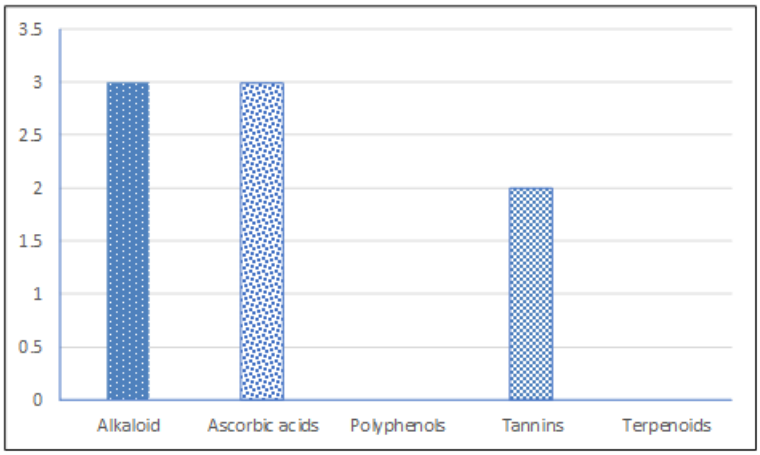

Fig 2: Histochemical test of *B. suberosa.*

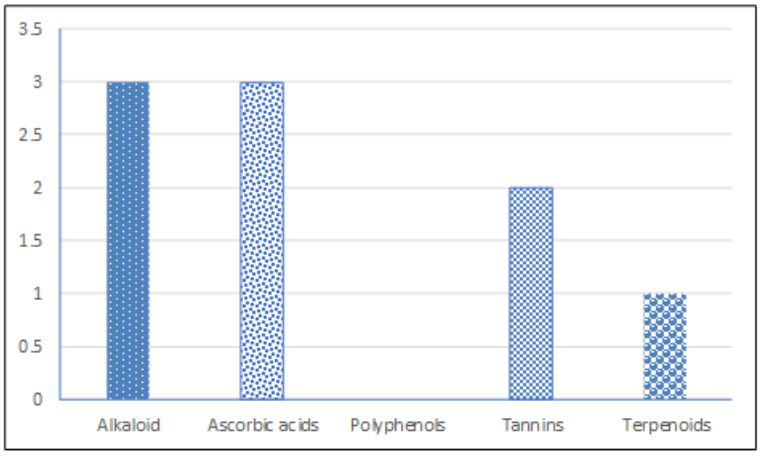

Fig.3: Histochemical test of *B. diffusa*

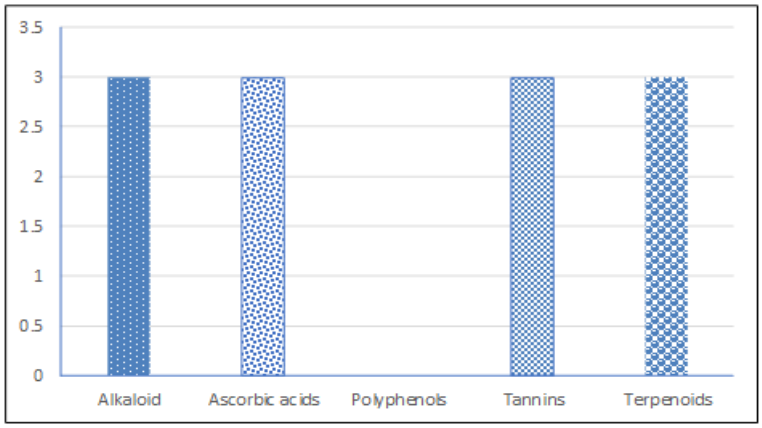

Fig 4: Histochemical test of *N. arbor-tristis.*

Table 2: Antibacterial activity of plant leaf extract.

Plants	Volume of Plant Extract (ml)	Antibacterial activity of Leaf Extract			
		B. subtilis	***K. pneumoniae***	***P. vulgaris***	***S. pneumoniae***
A. aspera	2	+	+++	+	+++
B. suberosa	2	++	+	+	+
B. diffusa	2	+	+++	–	+++
N. arbor-tristis	2	+	++	+++	+

+++ : 75% growth inhibition
++ : 50% growth inhibition
+ : 25% growth inhibition
– : No growth or Total inhibition

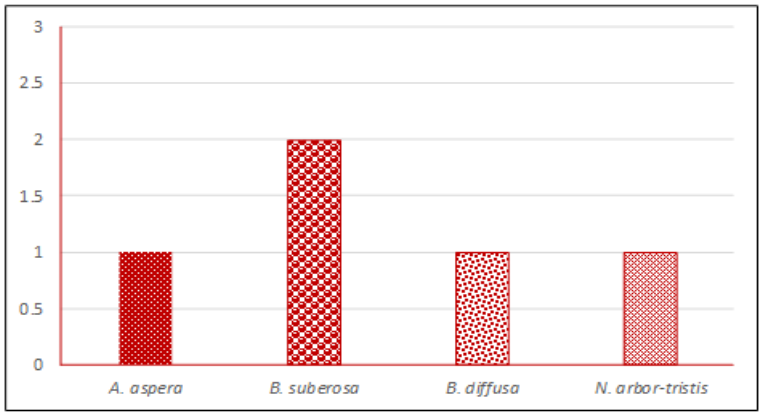

Fig.1: Antibacterial activity of *B. subtilis*

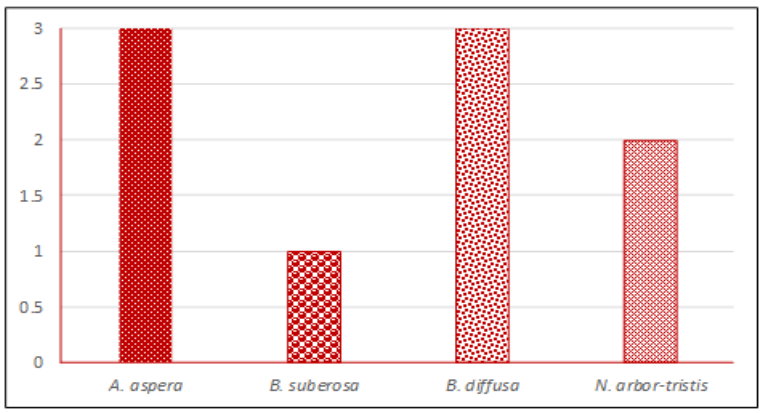

Fig.2: Antibacterial activity of *K. pneumoniae*

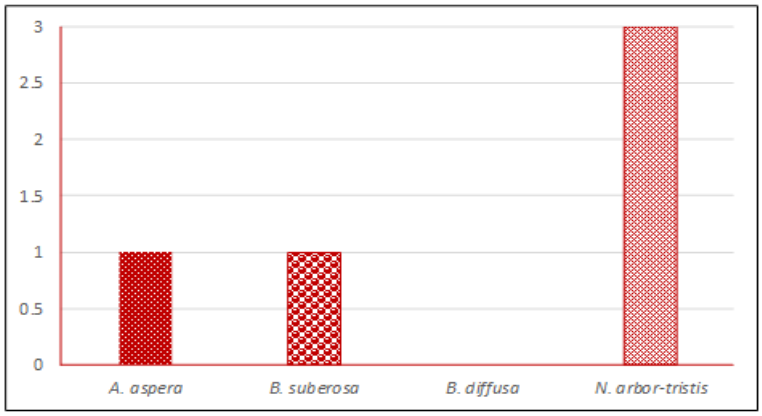

Fig 3: Antibacterial activity of *P. vulgaris.*

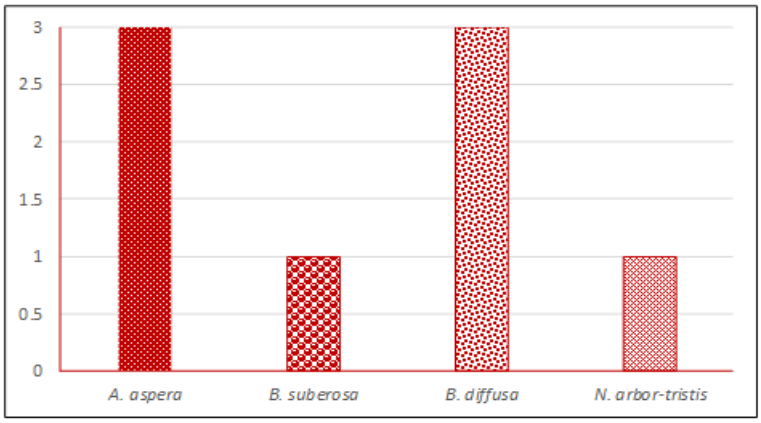

Fig 4: Antibacterial activity of *S. pneumonia.*

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 isolation, identification and purified compounds will serve as antibacterial phytochemical drug in pharmaceutical industries.

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