ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 5, Issue, 03, pp. 643-648, March, 2013

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

PHARMACOGNOSTIC STUDIES OF *Ampelocissus latifolia* (ROXB.) PLANCH – AN IMPORTANT ETHNOMEDICINAL PLANT

***Sutapa Choudhury, Chowdhury Habibur, Rahaman and Sudhendu Mandal**

Department of Botany Visva-Bharati Santiniketan-731235, India

ARTICLE INFO

Article History:
Received 17th December, 2012
Received in revised form
19th January, 2013
Accepted 14th February, 2013
Published online 19th March, 2013

Key words:
Ampelocissus latifolia,
Pharmacognostic features,
Epidermal micromorphology,
Antimicrobial activity.

ABSTRACT

The evaluation of quality and purity of crude drugs by means of various parameters is the most important aspects of pharmacognosy. The present study deals with different pharmacognostic parameters of *Ampelocissus latifolia* (Roxb.) Planch, an ethnomedicinally important plant of the family Vitaceae. The common name of the plant is 'Jungli angur'. Juice of tender leaves used in dental problems and as a detergent for indolent ulcers. The plant bears hypostomatic leaves and stomata are mainly anomocytic with few anisocytic types. Palisade ratio is 4 and stomatal index is 9. Needle shaped Ca-oxalate crystals are present on both epidermal surfaces of the leaf. In the methanolic extract of the leaf, the detected phytochemical groups are alkaloids, reducing sugars, gums, tannins and anthraquinones, etc. Ash value and moisture content of the leaf are 31.23% and 77.09 % respectively. The drug powder treated with different chemical reagents gives characteristic colourations when seen under UV light. This plant seems to be very potent against different popular bacteria. This study will throw new data regarding the uses of ethnomedicine to the state as well as national level inventory of ethnomedicine.

INTRODUCTION

Medicinal plant research has now got a momentum among the scientists of the world. The scientific evaluation of ethnomedicinally important plants is now being done thoroughly covering various aspects of their study like efficacy of the crude drugs, chemistry of active principles, different pharmacognostic parameters, etc. Chemical analyses and biological assay of medicinal plants are the important factors for identification of novel bioactive phytochemicals and drug discovery. Use of micromorphology and anatomy is now a recognised tool in the field of plant systematics. Importance of epidermal characters in general and those of trichomes in particular are widely recognised in taxonomic consideration of angiosperms (Banerjee *et al*., 2002; Mukherjee *et al*., 2000; Ogundipe and Olatungi, 1991; Parveen *et al.*, 2000; Raja Shanmukha Rao and Ramayya, 1987**)**. Ontogeny and structure of stomata are now also considered as an important taxonomic character for many of the angiospermic taxa (Ahmed, 1979; Carpenter and Smith, 1975; Inamdar, 1970; Kothari and Shah, 1975; Paliwal, 1966; Pant and Mehra, 1963; Rajagopal, 1979). The members of different genera and families of angiosperm have been studied anatomically by various workers with special emphasis on leaf epidermal micromorphology (Carlquist, 1961; Hagarup, 1953; Hossian and Khan, 1994; Kothari and Shah, 1975; Krishnamurthy and Kannabiran, 1970; Metcalfe and Chalk, 1950; Ogundipe and Akinrinlade, 1998). Only to some extent, the ontogeny, structure of stomata and phytochemical studies of different members of Vitaceae have been studied by different workers. But the detailed foliar epidermal characters including trichomes, stomata, chemical analysis, physical evaluation, antimicrobial activity, etc. of many members of the family Vitaceae have not yet been studied. Chemical analysis and biological assays are very important aspects in pharmacognostic evaluation of medicinal plants (Trease and Evans, 1983). Therefore, in this investigation the micromorphology of leaf epidermis, phytochemical screening and physical evaluation, antimicrobial activity of *Ampelocissus latifolia*, an ethnomedicinally important member of the family Vitaceae have been done. This investigation will be an useful marker for identification of the crude drugs obtained from the investigated taxa.

Corresponding author: smandalbot@gmail.com, habibur_cr@yahoo.co.in, sutapa.sain@rediffmail.com

MATERIALS AND METHODS

***Ampelocissus latifolia* (Roxb.) Planch. (Family: Vitaceae)**

Common name : Jungli angur, Gobralata, Govila, Icer.
Botanical Characteristics: Large twining herbaceous climbers with perennial rootstock. Leaves broadly orbicular-cordate, 3-5-7 angled or shallowly lobed, dentate, glabrous. Flowers in pyramidal paniculate compact cymes, reddish brown; peduncles with 2-branched slender tendrils. Calyx truncate. Petals oblong. Berries ellipsoid, black, 2 or rarely 3-4 seeded. Seeds ellipsoid, stony.

Flowering and fruiting time: September to June.
Distribution: Major parts of India**;** Kumaon to Moradabad, Assam, Konkan to Coromandal. Bangladesh (Sylet).
Habit and habitat: Large twining, annual, herbaceous climber. Terrestrial, wild, common in open waste lands, shrubberies and hedges.

Parts Used: Leaves and roots.
Medicinal uses: Leaves- juice of tender leaves used in dental problems and as a detergent for indolent ulcers. Leaves also used in cough and skin disease. Roots- paste applied to wounds; decoction given in dysentery.
Chemical constituents: Alkaloids, various types of saponins and anthraquinones, etc.

METHODS

For the study of foliar epidermis, leaf samples were cleared following the Bokhari's method (1970). The cleared leaf samples were then mounted on the slide with a drop of 10% glycerine and 1% aqueous safranin solution and observed under compound light microscope. The drawings of the leaf epidermal micromorphological characters were made with the camera lucida and measurements were taken with standardized ocular micrometer in each cases. Finally, the leaf powders were extracted (soxhlet extraction) with 90% methanol and these extracts were used for different chemical colour reaction tests for identification of different phytochemical groups. Screening of antimicrobial activity was carried out by agar diffusion method (Kirby-Bauer, 1966). 50 µl of plant extract was poured in 6mm wells punched in test culture seeded assay plates. Selected common human pathogenic bacterial strains like *Bacillus subtilis* (ATCC 11778), *Enterococcus faecalis (*ATCC 19433), *Escherichia coli* (NCTC 10418), *Klebsiella aerogenes* (W70), *Micrococcus luteus* (NCTC2665), *Pseudomonas aeruginosa* (NCTC10662), *Salmonella typhi* NCTC 19430 and *Streptococcus faecalis* MTCC 439) were cultured in nutrient broth of 24h old were used to seed the assay plates containing Nutrient Agar Media (NAM). Assay plates seeded with bacterial cultures were incubated at 37°C for 24 hours. After incubation antimicrobial activity was determined by measuring the zone of inhibition.

Macromorphology

Leaf: Herbaceous, broadly orbicular-cordate, 3-5-7 angled or shallowly lobed, dentate, glabrous, dark green above, light green beneath.
Stem: Large twining climber, branched, week, hollow, glabrous, dark green with brownish purple tint.

Micromorphology

General description and measurement of the epidermal cells, stomata, trichomes and crystals of the investigated plant have been represented in Tables 1, 2, 3 and 4.

Epidermis

Epidermal cell shape is irregular. Epidermal cell wall outline of the upper leaf surface is nearly straight and on the lower surface walls are wavy. The size of the upper epidermal cell is 19.98 µm x 39.96 µm and 19.98 µm x 34.96 µm on the lower surface. Epidermal cell frequency is 1530.83 /mm^2 on the upper surface and 1740.08 /mm^2 on the lower surface. Palisade ratio is 4 (Table 1, Fig. I- A, B, C, D, E, F, G, H, K).

Stomatal Complex

Leaves are hypostomatic i.e. stomata are exclusively present on the lower surface. Anomocytic with few anisocytic type of stomata are present on the lower surface. The size of the stomata is 23.31µm x 13.32 µm and frequency is 165.19 /mm^2. Stomatal index is 9 (Table 2, Fig. I- D, E, F, H).

Table 1. Foliar epidermal cell characters of the investigated plant

Plant	Leaf Surface	Cell Shape	Cell Length (µm)	Cell Width (µm)	Cell Frequency /mm^2	Cell Wall Outline	Palisade Ratio
Ampelocissus latifolia	Upper	Irregular	19.98	39.96	1530.83	Slightly straight	
	Lower	Irregular	19.98	34.96	1740.08	Wavy	4

Table 2. Stomatal features of the investigated plant

Plant	Leaf Surface	Stomatal Type	Stomatal Length (µm)	Stomatal Width (µm)	Stomatal Index (%)	Stomatal Frequency /mm^2
Ampelocissus latifolia	Upper	Absent	-	-	-	-
	Lower	Mainly anomocytic type; few anisocytic type	23.31	13.32	9	165.19

Table 3. Trichome features of the investigated plant

Plant	Leaf Surface	Types	Trichome Length (µm)	Trichome Width (µm)	Trichome Frequency /mm^2	Trichome Index %
Ampelocissus latifolia	Upper	Nonglandular unicellular with pointed apex	66.60	24.98	49.55	3
	Lower	Nonglandular unicellular with pointed apex.	66.60	24.98	49.55	
		Nonglandular uniseriate, bi-celled	99.90	14.43	27.53	

Table 4. Crystal features of the investigated plant

Plant	Surface	Types	Dissolved in
Ampelocissus latifolia	Upper	Needle Shaped	HCl
	Lower	Needle Shaped	HCl

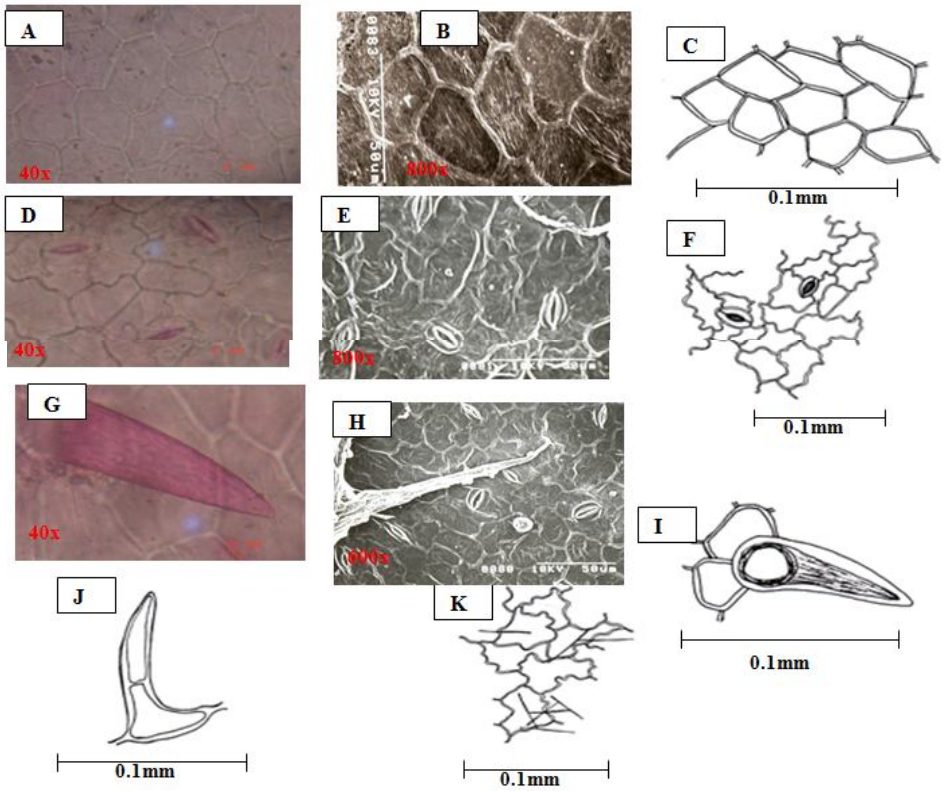


Fig. 1. Epidermal Micromorphology: A, B, C– Upper Epidermis without Stomata; Lower epidermis: D, E - Anomocytic Stomata; F- Anomocytic and Anisocytic Stomata; G, H, I, J- Nonglandular Trichomes; K - Needle Shaped Crystals

Trichomes

Nonglandular, unicellular trichomes with pointed apex are present on both upper and lower surfaces. Size is 66.60 µm x 24.98 µm and frequency is 49.55 /mm^2. In the upper surface nonglandular, uniseriate, bi-celled trichomes are also present. Size is 99.90 µm x 14.43 µm; frequency is 27.53 /mm^2**.** Trichome index is 3 (Table 3, Fig. I- G, H, I, J).

Crystals

Needle shaped Ca-oxalate crystals are present on both epidermal surfaces of the leaf (Table 4; Fig. I- K).

Organoleptic Features of the Crude Drug

Colour: Moderate yellow green; Odour: Characteristic; Taste: No specific taste; Texture: Herbaceous, glabrous, hairy (in fresh form).

Table 5. Microchemical tests of the leaf extract

Tests/ Reagents	Tests For	Nature of Changes	Leaf
Dragendroff's reagent	Alkaloids	Orange brown ppt	+
Wagner's reagent	Alkaloids	Orange brown ppt	+
Shinoda's tests	Flavonoids	–	–
10% NaOH	Flavonoids	–	–
Salkowski test	Steroids and triterpenoids	–	–
Benedict's reagent	Reducing sugars	Brick red ppt	+
Fehling's reagent	Reducing sugars	Brick red ppt	+
Molish's test	Gums	Red-violet ring	+
10% aqueous potassium dichromate solution	Tannins	Yellowish-brown ppt	+
10% aqueous lead acetate solution	Tannins	Yellow ppt	+
5% aqueous ferric chloride solution	Tannins	Greenish-black colour	+
1% lead acetate	Saponins	–	–
Borntrager's test	Anthraquinones	Pink colour	++

- = Absent; + = Present

Table 6. UV fluorescence nature of the investigated plant

Materials and treatment	In fluorescence light	In ordinary light
Powder as such	Light green	Moderate yellow green
Treated with dilute nitric acid	Yellow	Dark olive green
Treated with sodium hydroxide in water	Deep pink	Red
Treated with hydrochloric acid	Strong pink	Light orange
Treated with dilute sulphuric acid	Very dark greenish blue	Blue
Treated with antimony trichloride	Light brown	Brown

Microchemical Evaluation of the Powdered Drug

Through the phytochemical tests of the methanolic extract of leaf, the detected phytochemical groups are alkaloids, reducing sugars, gums, tannins and anthraquinones, etc. (Table 5).

Physical Evaluation

PHYSICAL CONSTANT

Ash Value

a) Total ash - 31.23%
b) Water soluble ash - 11.84%
c) Acid insoluble ash - 09.70%

Moisture Content - 77.09 % (in fresh form).

FLUORESCENCE ANALYSIS

Here in this study it is observed that drug powder treated with different chemical reagents gives characteristic colourations when seen under UV light and it is compared with the colourations observed under ordinary light. In some cases there are marked differences in colour (Table 6).

Antimicrobial activity

Methanolic foliar extract of *Ampelocissus latifolia* was inhibitory against *Bacillus subtilis (*ATCC 11778), *Enterococcus faecalis* (ATCC 19433), *Klebsiella aerogenes* (W70), *Micrococcus luteus* (NCTC 2665), *Pseudomonas aeruginosa (*NCTC 10662) and *Salmonella typhi* (NCTC10662); whereas ethyl acetate soluble extract was inhibitory to *Bacillus subtilis* (ATCC 11778), *Klebsiella aerogenes* (W70), *Micrococcus luteus* (NCTC 2665), *Pseudomonas aeruginosa* (NCTC 10662) and *Salmonella typhi* (NCTC 19430). Only water soluble extract showed no inhibition zone against the selected bacteria. It is found that methanolic foliar extract exhibited maximum inhibitory effect to *Enterococcus faecalis* (inhibition zone, 24mm) and ethyl acetate foliar extract showed highest activity against *Pseudomonas aeruginosa* (inhibition zone, 18mm) (Table 7).

Leaf micromorphology of this investigated plant show the general features of *Ampelocissus latifolia* which conform to the characters reported in earlier works (Metcalfe and Chalk, 1950, 1979). Importance of epidermal characters in general is widely recognized in taxonomic considerations of angiosperm (Rao, 1987; Stace, 1965 a,b) and in many cases they have successfully used in identification of taxa at genus as well as species levels. Stomata of *Ampelocissus latifolia* are mainly anomocytic type. Studies in stomata can have a great taxonomic as well as pharmacognostic value in proper identification of different plant taxa including medicinal plants (Inamdar, 1970; Kothari and Shah, 1975; Krishnamurthy and Kannabiran, 1970; Pant and Mehra, 1963; Trease and Evans, 1978, 1983). Size of the stomata is 23.31µm x 13.32 µm on the lower surface. Frequency of the stomata is 165.19 /mm^2 on the lower surface. Trichome features are also very important in proper identification of the plants and considered as one of the valuable taxonomic marker now (Leelavathi and Ramayya, 1983). Here nonglandular type of trichomes is found. Needle shaped crystals are present.

In case of crude drug evaluation, ash value plays a very important role which includes % of total ash, water soluble ash and acid insoluble ash (IP, 1996). Ash value gives a maker character for identification of crude drugs obtained from the investigated taxa. Here ash value of *Ampelocissus latifolia* is 31.23% which is very distinct and will be successfully used in evaluation of drug quality of the plant. Similarly fluorescence characters of the crude drugs are considered very important marker in making distinction among the drugs. Here some fluorescence features have been identified which are very much distinctive in identifying the respective drug obtained from this plant. Resistance to antimicrobial agents is emerging in a wide variety of microorganisms and multiple drug resistant organisms pose serious threat to the treatment of infectious diseases (Tomir and Tomasz, 1986). Keeping this problem in mind various workers are actively involved in bioprospection of phytochemicals as potent antimicrobials from the ethnomedicinal plants with the help of ethnobotanical leads (Cox and Balick, 1994; Favel, *et al.*, 1994; Hiremeth, *et al.*, 1996; Hostettmann and Marston, 1995; Majorie, 1999; Sukul and chaudhari, 2001). Kirby-Bauer (1966) elaborately showed the methods of detecting antimicrobial activity of certain specimens.

Table 7. Zone of inhibition (mm) for *Ampelocissus latifolia* leaf extracts against some gram-positive and gram-negative bacteria

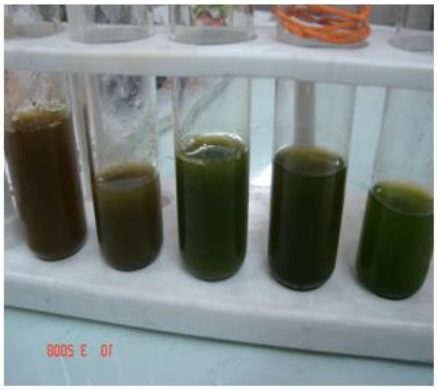
Selected Bacteia	Inhibition Zone (mm)								
	Methanolic extract			Water extract			Ethyl Acetate extract		
	24 Hrs	48 Hrs	72 Hrs	24 Hrs	48 Hrs	72 Hrs	24 Hrs	48 Hrs	72 Hrs
Bacillus subtilis ATCC 11778	10	11	12	-	-	-	12	12	12
Enterococcus faecalis ATCC 19433	24	24	24	-	-	-	-	-	-
Escherichia coli NCTC 10418	-	-	-	-	-	-	-	-	-
Klebsiella aerogenes W70	10	10	10	-	-	-	11	12	12
Micrococcus luteus NCTC2665	11	11	11	-	-	-	10	11	11
Pseudomonas aeruginosa NCTC10662	16	16	16	-	-	-	18	18	18
Salmonella typhi ATCC 19430	-	10	10	-	-	-	11	12	12
Streptococcus faecalis MTCC 439	-	-	-	-	-	-	-	-	-

DISCUSSION

Pharmacognosy implies a particular knowledge of methods of identification and evaluation of crude drugs obtained from plants which include macromorphology, phytochemical and pharmacological studies. The major problem of the commercial supply of crude drugs is identification of genuine drug. Crude drugs may easily be adulterated or substituted by or confused for other ones because neither it has any trade name printed on it nor it carries any identifying structure for the easy identification of it by the plant taxonomists, rather drug samples supplied are shrunked, rolled, twisted, deformed and discoloured. So, pharmacognostic evaluation of crude drugs with macromorphology, micromorphology, organoleptic tests, ash value, histochemical colour reactions and UV fluorescence study will help in identifying genuine drugs and thus in checking adulteration, because the tests are very specific for a particular drug.

Some Photographs of Chemical Colour Reaction Tests

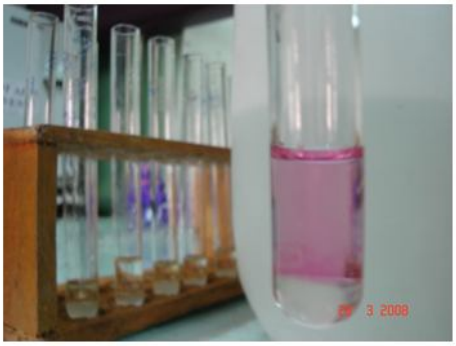
Soxhlet Apparatus (Extract done through it)



Leaf extract

Extract evaporating in water bath

Reducing sugars present in the leaf extract (Brick- red ppt)



Anthraquinones present in the leaf extract (Purple colour)

Recently floral and foliar extracts are screened by different workers against several pathogenic and non-pathogenic microorganisms (Jain *et al.*, 2006; Akhtar *et al*., 2006). In this context, antimicrobial activity of the selected plant has been done. Methanolic foliar extract of *Ampelocissus latifolia* was inhibitory to *Bacillus subtilis, Enterococcus faecalis, Klebsiella aerogenes, Micrococcus luteus, Pseudomonas aeruginosa* and *Salmonella typhi*; whereas ethyl acetate soluble extract was inhibitory to *Bacillus subtilis, Klebsiella aerogenes, Micrococcus luteus, Pseudomonas aeruginosa* and *Salmonella typhi*. Only water soluble extract showed no inhibition zone against the selected bacteria. It is found that methanolic foliar extract exhibited maximum inhibitory effect to *Enterococcus faecalis* (inhibition zone, 24mm) and ethyl acetate foliar extract showed highest activity against *Pseudomonas aeruginosa* (inhibition zone, 18mm). Finally this study will provide some distinctive pharmacognostic features by which the crude drugs obtained from the plant *Ampelocissus latifolia* will be properly identified and also the study will throw some scientific knowledge to herbalists and pharmacologists for proper evaluation of this folk drug.

Acknowledgements

We are very grateful to the Head of the Botany Department, Visva-Bharati for providing the necessary laboratory facilities and also very thankful to the University Grant Commission, Govt. of India for financial assistance.

REFERENCES

Ahmed, K.J. 1979. Stomatal features of Acanthaceae. In *Structure, Function and Ecology of Stomata* (ed. D.N. Sen). Bishen Singh Mahendra Pal Singh, Deheradun, India. pp. 43-60

Akhtar, N., Ali, M. and Das, A. 2006. Antimicrobial activity of *Inula cuspidata* leaves. *Indian J.Nat. Prod.* 22 (3): 33-34.

Banerjee, A., Rahaman, C. H., Kar, R.K. and Mandal, S. 2002. Micromorphology of foliar epidermis of some tropical tree legumes. *Phytomorphology* 52(2 and 3): 223-230.

Bauer, A.W., Kirby, W.M.M., Sherris, J.C. and Truck, M. 1966. Antibiotic susceptibility testing by a standardized single disc method. *Am. J. Clin. Pathol.* 45: 493-496.

Bokhari, M.H. 1970.: Morphology and Taxonomic significance of foliar sclereids in *Limonium* . *Notes Royal Bot. Gard.* Edinburgh. 30: 43-53.

Carlquist, S. 1961. *Comparative Plant Anatomy*, Holt Rinechart and Winston, New York, USA.

Carpenter, S.B. and Smith, N.D. 1975. Stomatal distribution and size in Southern Appalachian hardwoods. *Can. J. Bot.* 53: 1153 – 1156.

Chakravarty, H.L. 1937. Physiological anatomy of leaves of Cucurbitaceae. *Philipp. J. Sci.* 63 (4): 409-431.

Cox, P.A., and Balick, M.J. 1994. The ethnobotanical approach to drug discovery. *Sci. Am* 270: 82-87.

Favel, A., Steinmetz, M.D., Regli, P., Olivier, E.V., Elias, R. and Balansard, G. 1994. In vitro antifungal activity of triterpenoid saponins. *Planta Med.* 60: 50-53.

Hagarup, O. 1953.: The Morphology and Systematics of the leaves of Ericales. *Phytomorphology* 3: 459 – 464.

Hiremeth, S.P., Swamy, H.K.S., Shishailappa, B. and Meena, S. 1996. Antibacterial and antifungal activities of *Striga densiflora* and *Striga orobanchioides. Indian J. Pharm. Sci.* 174-176.

Hossian, A.B.M.E. and Khan, S.A. 1994. Use of epidermal character for identification of *Blumea* (Compositae) species from Bangladesh. *Bang. J. Bot.* 23: 147 – 154.

Hostettmannn, K. and Marston, A. 1995. *Saponins (Chemistry and Pharmacology of Natural Products)*. University Press, Cambridge.

Inamdar, J.A. 1970. Epidermal Structure and Ontogeny of Caryophyllaceous stomata in some Acanthaceae. *Bot. Gaz.* 131: 261-268.

Indian Pharmacopoeia (IP). 1996. 4th edn.Vol. 1, The Controller of Publications, New Delhi. pp. A 100-A124.

Jain, P.K., Belsare, D.P., Mandal, S.C., Pal, S.C., Badgujar, V.B., and Surana, S.J. 2006.: Antimicrobial activity of stem bark of *Kigelia piñata*. *Indian. J. Nat. Prod.* 22 (3): 30-32.

Johansen, D.A. 1940. *Plant Microtechnique*, Mc Graw-Hill, New York.

Kothari, M.J. and Shah, G.L. 1975. Epidermal structure and ontogeny of stomata in the Papilionaceae. *Bot. Gaz*. 136: 372-379.

Krishnamurthy, K.H. and Kannabiran, B. 1970. Histomorphology of foliar epidermis and pharmacognosy in Asclepiadaceae. *J. Indian Bot. Soc*. 49: 105-113.

Leelavathi, P.M. and Ramayya, N. 1983. Structure, distribution and classification of plant trichomes in relation to taxonomy. Caesalpinioidae. *Indian J. For.* 6: 43-56.

Majorie, M.C. 1999. Plant products as antimicrobial agents. *Clin. Microbiol. Rev.* 12(4): 564-582.

Metcalfe, C.R. and Chalk, L. 1979. *Anatomy of Dicotyledons.* Vol 1, 2nd edn, Clarendon Press, Oxford, UK.

Metcalfe, C.R. and L. Chalk 1950. *Anatomy of Dicotyledons*. Vol 2, Clarendon Press, Oxford, UK.

Mukherjee, K.K., Roy, M. Saha, P.K. and Ganguly, S.N. 2000. Surface morphology of tea (*Camellia sinensis* L.) leaves. *Phytomorphology* 50: 125-131.

Ogundipe, O.T. and Akinrinlade, O. 1998. Epidermal micromarphology of some species of *Albizia* Durazz (Mimosaceae). *Phytomorphology* 48: 325 – 333.

Ogundipe, O.T. and Olatunji, O.A. 1991. The leaf anatomy of the species of *Cochlospermum* Kunth (Cochlospermaceae) in West Africa. *Feddes Repertorium* 102: 183-187.

Paliwal, G.S. 1966. Structure and ontogeny of stomata in some Acanthaceae. *Phytomorphology* 16: 533-539.

Pant, D.D. and Mehra, B. 1963. Development of Caryophyllaceous stomata in *Asteracantha longifolia* Nees. *Ann. Bot.* 27: 647-652.

Parveen, N.S. Murthy, K.S.R. and Pullaiah, T. 2000.: Leaf epidermal characters in *Crotalaria* sp. (Papilionoideae) from Eastern Ghats. *Phytomorphology* 50: 205-212.

Rajagopal, T. 1979. Distributional patterns and taxonomic importance of foliar stomata. *Indian J. Bot.* 2: 63-69.

Rao, R.S. and Ramayya, N. 1987. Trichome types and their taxonomic importance in the Tiliaceae. *Indian J. Bot.* 10: 65-73.

Stace, C.A. 1965a. Cuticular studies as an aid to Plant Taxonomy. *Bull. Br. Mus. Nat. Hist. Botany* 4: 3-78.

Stace, C.A. 1965b. The taxonomic importance of the leaf surface. In *Current Concepts of Plant Taxonomy* (eds. V.H. Heywood and D.F. Moore). Academic Press, London. pp. 67-94.

Sukul, S. and Chaudhari, S. 2001. Antimicrobial natural products from leaves of *Lantana camara* with activity comparable to some therapeutically used *antibiotics*. *Ind. J. Pharm. Sci.* 63: 20-23.

Tomir, E. and Tomasz, A. 1986. β- lactum specific resistant mutant of *Staphylococcus aureus*. *Antimicrob. Agents. Chemother*. 30: 577-583.

Trease, G.E. and Evans, W.C. 1983. *Pharmacognosy,* 12th edn. English Language Book Society / Bailliere Tindall.

****