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Phytochemical Screening and Antibacterial Studies of the Leaf Extract of *Eurya japonica* Thunb. and *Ficus auriculata* Lour.

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Medicinal plants are of great importance to the health of individuals and the society. The medicinal value of the plants lies in some chemical substances that produce a definite physiological action on the human body .In this study we examined the phytochemical screening and antibacterial activities of the leaf of *Eurya japonica* and *Ficus auriculata* plant species. Phytochemical screening of these plants was performed for constituents i.e alkaloids, glycosides, flavonoids , terpenoids,saponins, tannins and reducing sugar using four (4) different solvents namely petroleum ether, ethyl acetate, acetone and ethanol respectively. Thier antibacterial activities were tested using some Gram positive bacteria: *(Staphylococcus aureus)* and Gram negative bacteria (*Escherichia coli* ,*Klebseila pneumonia* and *Pseudomonas* species) by disc diffusion method. The disc diffusion method for antibacterial activity showed significant reduction in the bacterial growth in terms of zone of inhibition around the discs. The results of the antibacterial activity screening support the ethno-medical use of these plants. Further studies on the isolation and characterization of the compound from *Eurya japonica* and *Ficus auriculata,* responsible for the observed antibacterial properties is in progress.

Key words: Antibacterial activity, ethanolic extracts, *Eurya japonica, Ficus auriculata,* medicinal plants and phytochemical screening.

Medicinal plants are of great importance to the health of individuals and the society. The medicinal value of the plants lies in some chemical substances that produce a definite physiological action on the human body (Stephan *at al*, 2009). Medicinal plants also represent a rich source of antimicrobial agents. Plants are used medicinally in different countries and are rich source of many potent and powerful drugs. The use of plants as antibacterial agents is gradually attracting attention probably due to the high cost, unavailability and resistance of the drugs (Fransworth and Morris, 1976). *Eurya* is a genus of dioecious shrubs and trees (separate female and male plants) native to southern and eastern Asia and the Pacific Islands. As an ornamental plant, *Eurya japonica* has much to recommend it, with its strong herringbone branching pattern, dark green leaves (turning burgundy in winter), copious greenish-white flowers and, on female plants, black berries. The flowers are attractive, but smell somewhat metallic and bile-like. Branches of *Eurya japonica* are used in Shinto ceremonies in Japan (http://www.ubcbotanicalgarden.org/education/eurya.php).

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The leaves are used as a substitute of tea or to adulterate China tea (Facciola, 1979). *Eurya* is the largest genus in the Ternstroemiaceae. Chi-Chih *at al*, studied the Phylogeny and taxonomy of *Eurya* (Ternstroemiaceae) from Taiwan, as inferred from ITS sequence data. *Eurya japonica Thunb.* (local name –Baunra , family – Theaceae) is a shrub and its leaves are used as poultice on skin eruption (http://senapati.nic.in/FloraandFauna.html).

Ficus auriculata (Roxburgh Fig) - This is an evergreen to semi-deciduous, spreading large shrub or small tree reaching 25 feet tall and as wide. Fruits are edible and can be made into jams and curries. Leaves are lopped for fodder. Munesh Kumar and Vishwapati Bhatt (2006) in their studies stated that *Ficus auriculata* can be taken as fodder and fiber. Roder *at al(2003)*, stated *Ficus auriculata* is an important tree fodder in the Himalayan region of Nepal and India and its imperative to carry out research exploring its potential in evolving production systems and to quantify the opportunities of improving its nutritional quality and productivity through selection. Detailed studies about *Ficus auriculata* has not been done so far. However Sirisha (2010) studied the antioxidant properties of *Ficus* species on this he studied the Bark, root, leaves, fruit and latex of some selected ficus species which are frequently used for the treatment of various ailments.

Correlating these two plants in terms of their medicinal properties through their traditional knowledge, the principle aim of the present work was to study the phytochemical properties and the antibacterial activity of *Eurya japonica* and *Ficus auriculata*.

MATERIAL AND METHODS

The leaves of *Eurya japonica* and *Ficus auriculata* plants were collected randomly for their phytochemical and the antibacterial studies respectively from the Phayeng village of Imphal West districtl,Manipur,India. The collected plant materials were botanically authenticated by Botanical Survey of India, Shillong.

Preparation of extracts

The powdered sample (100g) was weighed and subjected to soxhlation with petroleum ether for 72 hrs. Similarly, the procedure was repeated with ethyl acetate, acetone and ethanol as solvents, using 100g of the fresh ground sample, for each extraction .The solvents were distilled off at lower temperature under reduced pressure in the rotary evaporator and concentrated to dryness.

Phytochemical screening

Phytochemical screening was performed using standard procedures

Test for alkaloids

The presence of an alkaloid is ascertained by treating plant extract with various alkaloidal reagents such as potassium mercuric iodide (Mayer's reagent), iodine dissolved in potassium iodide (Wagner's reagent), saturated picric acid aqueous solution(Hager's reagent).2ml of each extract was evaporated to dryness and residue was heated on a boiling water bath with 2N HCL (5 ml). After cooling the mixture was filtered and the filtrate was divided into three equal portions. One portion was treated with a few drops of Mayer's reagent, the other with Harger's reagent and the last with equal amount of Wagner's reagent (Rizk, 1982). The sample was then observed for the presence of turbidity or precipitation.

Test for carbohydrates (Molish test)

To 2 ml of the filtrate 2 ml distilled water was added and filtered. To the filtrate few drops of Molish reagent was added .The appearance of violet or reddish violet colour indicates the presence of carbohydrates.

Test for reducing sugars (Fehling's test)

To 0.5 ml of extract solution, 1 ml of water was added and 5-8 drops of Fehling's solution (A and B) was added at hot in a test tube. The solution was observed for brick red precipitate.

Test for Glycosides (Keller-kiliani test)

To 2 ml of the filtrate, 1 ml of glacial acetic acid was added followed by few drops of $FeCl_3$ solution and conc. H_2SO_4.The development of green-blue color indicates the presence of cardiac glycosides.(Oguyemi *et al*., 1979)

Test for tannins

To 0.5 ml of extract solution 1 ml of water and 2 drops of ferric chloride solution was added. Blue color was observed for gallic tannins and green black for catecholic tannins.(Iyengar,1995)

Test for flavonoids

To 5 mg of each extract was treated with

a few drops of conc. 2N HCL and Magnesium turnings (0.5 g). The presence of Flavonoids was indicated if pink or magenta red colour developed within 3 min (Somolenski *et al*., 1972).

Test for saponins (Salkowski test)

To 20 mg of extract was teated with 2.5 ml of acetic anhydride and 2.5 ml of chloroform . then concentrated solution of sulphuric acid was added slowly and red violet color was observed for terpinoid and green bluish color for steroids.(Siddiqui and Ali,1997).

Antibacterial studies

Test organisms

The extracts were tested on the following Gram positive bacteria: *Staphylococcus aureus* and Gram negative bacteria: *Escherichia coli*,

Table 3.1. Preliminary phytochemical test of *Eurya japonica*

S. No	Tests	Petroleum ether	Ethyl acetate	Methanol	Acetone	Ethanol
1.	Alkaloid test :					
	Mayer's test	+ ve	+ ve	+ ve	+ ve	+ ve
	Harger's test	+ ve	+ ve	+ ve	+ ve	+ ve
	Wagner's test	+ ve	+ ve	+ ve	+ ve	+ ve
2.	Carbohydrates test :					
	A) Molish's test	+ ve	+ ve	+ ve	+ ve	+ ve
	B) Fehling's test	+ ve	- ve	+ ve	+ ve	+ ve
3.	Glycosides :					
	Keller-kiliani test	+ ve	+ ve	+ ve	- ve	- ve
4.	Tannins :					
	Aqueous $FeCl_3$ test :	+ ve	+ ve	+ ve	+ ve	+ ve
5.	Flavonoids :					
	Mg/ HCl test :	- ve	-ve	+ ve	+ ve	+ ve
6.	Saponin :					
	Foam test :	+ ve	-ve	+ ve	+ ve	-ve
7.	Steroids :					
	Salkawaski test :	+ ve	-ve	+ ve	+ ve	+ ve

Table 3.2. Preliminary phytochemical test of *Ficus auriculata*

S. No	Tests	Petroleum ether	Ethyl acetate	Methanol	Acetone	Ethanol
1.	Alkaloid test :					
	Mayer's test	+ ve	+ ve	+ ve	+ ve	
	Harger's test	+ ve	+ ve	+ ve	+ ve	
	Wagner's test	+ ve	+ ve	+ ve	+ ve	
2.	Carbohydrates test :					
	A) Molish's test	+ ve	+ ve	+ ve	+ ve	
	B) Fehling's test			- ve	+ ve	
3.	Glycosides :					
	Keller-kiliani test	+ ve	+ ve	+ ve	+ ve	
4.	Tannins :					
	Aqueous $FeCl_3$ test :	+ ve	+ ve	+ ve	+ ve	
5.	Flavonoids :					
	Mg/ HCl test :	- ve	-ve	+ ve	+ ve	
6.	Saponin :					
	Foam test :	- ve	- ve	- ve	-ve	
7.	Steroids :					
	Salkawaski test :	- ve	-ve	- ve	+ ve	

Table 3.3. Antibacterial activity of *Eurya japonica*

S. No.	Test organisms	Concentration (mg/ml) 50mg /ml	100mg/ml	150mg/ml	200mg/ml	Test control	Standard control (10µg/disc) Strepto-mycin	Chloram-phenicol	tetracyclin	Norfloxacin
Petroleum ether extract										
1.	*Staphylococcus sp.*	6.33	7.66	8.33	8.66	0.00	19.33	21.66	22.66	18.00
2.	*Klebseila sp.*	6.66	7.33	8.33	9.33	0.00	11.00	17.00	15.33	25.33
3.	*E. coli*	6.66	8.66	9.66	12.00	0.00	16.66	14.00	7.33	22.66
4.	*Pseudomonas sp.*	6.66	7.33	8.00	9.00	0.00	16.33	15.66	9.33	9.00
Ethyl acetate extract:										
1.	*Staphylococcus sp.*	7.33	8.33	8.66	9.33	0.00	19.33	21.66	22.66	18.00
2.	*Klebseila sp.*	6.00	8.00	9.66	13.33	0.00	11.00	17.00	15.33	25.33
3.	*E. coli*	6.00	6.66	7.66	9.00	0.00	16.66	14.00	7.33	22.66
4.	Pseudomonas sp.	6.33	7.66	9.00	9.66	0.00	16.33	15.66	9.33	9.00
Acetone extract:										
1.	*Staphylococcus sp.*	8.33	9.33	10.33	12.33	0.00	19.33	21.66	22.66	18.00
2.	*Klebseila sp.*	7.33	8.00	11.00	12.00	0.00	11.00	17.00	15.33	25.33
3.	*E. coli*	7.33	8.66	10.66	12.66	0.00	16.66	14.00	7.33	22.66
4.	*Pseudomonas sp.*	6.33	8.66	11.00	13.00	0.00	16.33	15.66		9.33
9.00										
Ethanol extract:										
1.	*Staphylococcus sp.*	8.33	9.66	10.33	11.66	0.00	19.33	21.66	22.66	18.00
2.	*Klebseila sp.*	6.33	7.33	7.00	8.00	0.00	11.00	17.00	15.33	25.33
3.	*E. coli*	8.66	10.66	11.00	13.33	0.00	16.66	14.00	7.33	22.66
4.	*Pseudomonas sp.*	6.33	9.66	10.33	11.66	0.00	16.33	15.66	9.33	9.00

Table 3.4. Antibacterial activity of *Ficus auriculata*

S. No.	Test organisms	Concentration (mg/ml) 50mg /ml	100mg/ml	150mg/ml	200mg/ml	Test control	Standard control (10µg/disc) Strepto-mycin	Chloram-phenicol	tetracyclin	Norfloxacin
Petroleum ether extract										
1.	*Staphylococcus sp.*	-	-	-	-	0.00	19.33	21.66	22.66	18.00
2.	*Klebseila sp.*	6.00	6.00	6.00	7.00	0.00	11.00	17.00	15.33	25.33
3.	*E. coli*	-	-	-	-	0.00	16.66	14.00	7.33	22.66
4.	*Pseudomonas sp.*	6.00	6.00	6.00	7.00	0.00	16.33	15.66	9.33	9.00
Ethyl acetate extract:										
1.	*Staphylococcus sp.*	8.00	8.50	9.50	11.00	0.00	19.33	21.66	22.66	18.00
2.	*Klebseila sp.*	7.00	7.00	8.00	8.00	0.00	11.00	17.00	15.33	25.33
3.	*E. coli*	6.00	6.00	6.00	7.00	0.00	16.66	14.00	7.33	22.66
4.	*Pseudomonas sp.*	6.00	6.00	7.00	7.50	0.00	16.33	15.66	9.33	9.00
Acetone extract:										
1.	*Staphylococcus sp.*	-	6.00	7.00	8.00	0.00	19.33	21.66	22.66	18.00
2.	*Klebseila sp.*	6.00	6.50	6.50	7.00	0.00	11.00	17.00	15.33	25.33
3.	*E. coli*	6.00	6.50	7.50	8.00	0.00	16.66	14.00	7.33	22.66
4.	*Pseudomonas sp.*	6.00	7.00	8.00	9.00	0.00	16.33	15.66	9.33	9.00
Ethanol extract:										
1.	*Staphylococcus sp.*	6.33	7.33	8.33	9.00	0.00	19.33	21.66	22.66	18.00
2.	*Klebseila sp.*	6.50	7.00	7.00	8.00	0.00	11.00	17.00	15.33	25.33
3.	*E. coli*	6.00	6.00	7.00	8.00	0.00	16.66	14.00	7.33	22.66
4.	*Pseudomonas sp.*	6.00	7.00	8.00	9.00	0.00	16.33	15.66	9.33	9.00

Klebseila pneumonia and *Pseudomonas species*. All the organisms were procured from the Microbiology lab of Silchar Medical College, Silchar, Assam.

Disc diffusion method

The bioassay for bacterial strains was employed by disc diffusion method (Ergene *et,al* 2006). Filter paper discs (Watman

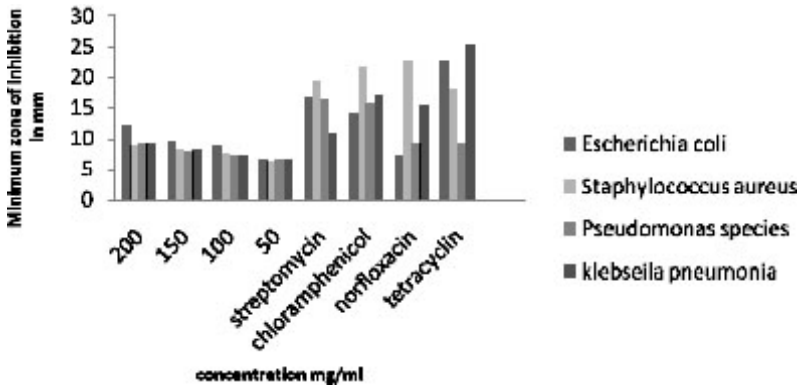

Fig. 1. Antibacterial activity of leaf extract of Eurya Japonica on petroleum ether extract

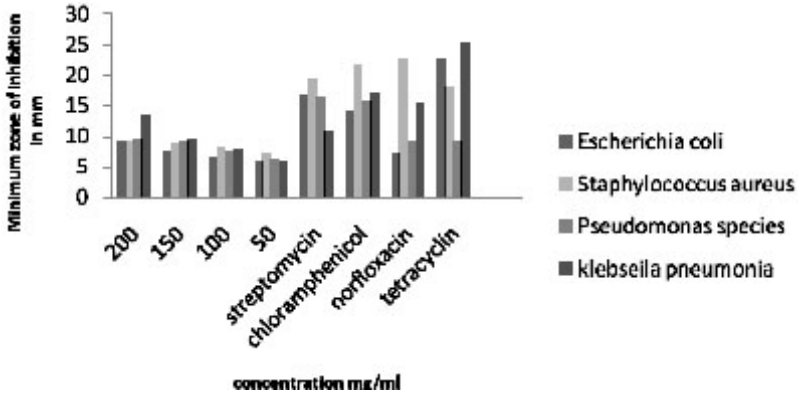

Fig. 2. Antibacterial activity of leaf extract of Eurya Japonica on ethyl acetate extract

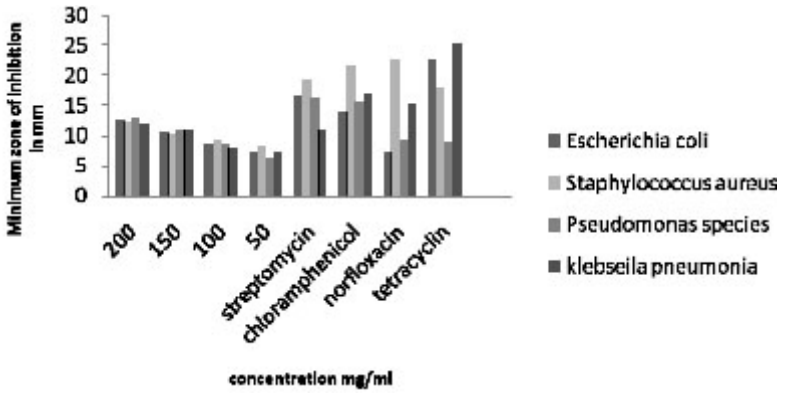

Fig. 3. Antibacterial activity of leaf extract of Eurya Japonica on acetone extract

No. 1) of 5 mm diameter was punched with paper puncher. The discs were then immersed with 20µl of the plant crude extract of different concentrations ranging from 50 mg/ml, 100mg/ml, 150mg/ml and 200mg/ml respectively. The disc were incubated for 24 hrs and then dried at room temperature. The dried disc were sterilised by keeping under ultraviolet radiation for 1 hour.

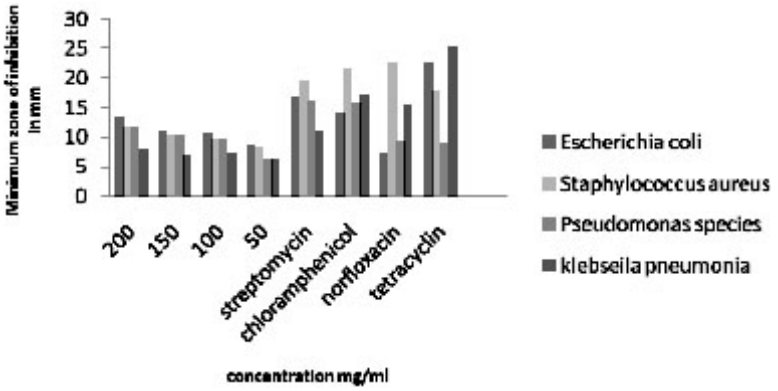

Fig. 4. Antibacterial activity of leaf extract of Eurya Japonica on ethanol extract

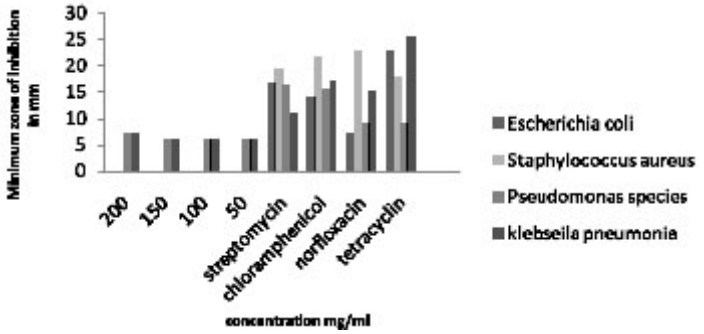

Fig. 5. Antibacterial activity of leaf extract of Ficus auriculata on petroleum ether extract

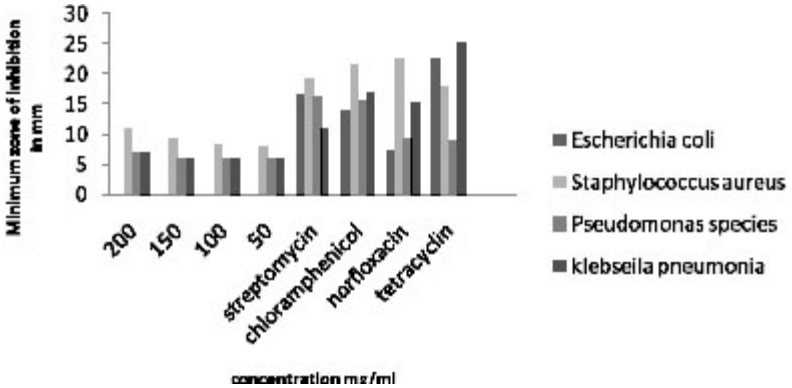

Fig. 6. Antibacterial activity of leaf extract of Ficus aruiculata on ethyl acetate extract

Preparation of media

Media were prepared using Nutrient agar of 14g, weighed accurately and dissolved in 500ml sterile double distilled water in a conical flask. The conical flask was plugged with cotton and sterilized by autoclaving in 15 psi at 120° C for 15 mins. This is then poured into petridish and cooled to solidify.

Antibacterial assay

Four different concentrations of the leaf extracts were tested for antibacterial activity using nutrient agar disc diffusion method. 10 ml nutrient agar was dispensed into Petri dishes and allowed to solidify. Bacteria were recovered by gently swabbing the surface of the culture plates with a sterile innoculation loop and the loop dipped in

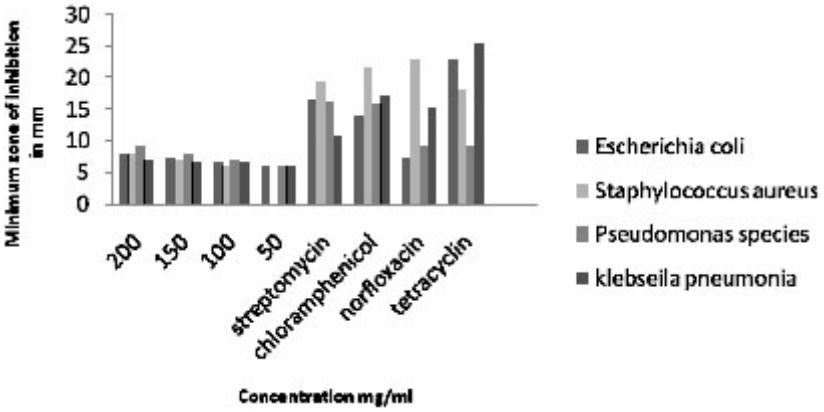

Fig. 7. Antibacterial activity of leaf extract of Ficus aruiculata on acetone extract

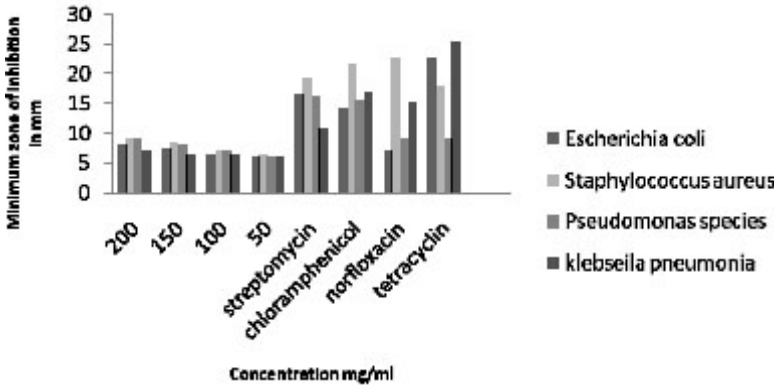

Fig. 8. Antibacterial activity of leaf extract of Ficus aruiculata on ethanol extract

5ml peptone water in a test tube to suspend the bacteria. Media which were poured in the petridish were inoculated with test organisms from the suspension using cotton swab. The sterile discs impregnated with 20µl of test extract were introduced onto the upper layer of the seeded nutrient agar plate. This was incubated at 37° C for 24 hrs. The diameter of zone of inhibition in mm was recorded after incubation. The experiment was performed in triplicate and average diameter of zone of inhibition was recorded. Streptomycin, Tetracycline ,Chloramphenicol and Norfloxacin (10µg/disc) were used as standard control. Test control was also prepared with different solvents (20 µl/ml).

DISCUSSION

Phytochemical screening of the plants showed the presence of alkaloids, glycosides, flavonoids , terpenoids,saponins, tannins and reducing sugar in *Eurya japonica* however only saponins was absent in *Ficus auriculata* plant species.The disc diffusion method for antibacterial activity showed significant reduction in the bacterial growth in terms of zone of inhibition around the dics. Among bacterial forms tested *Escherichia coli, Klebseila* and *Pseudomonas* were found to be more sensitive to the ethyl acetate ,acetone and ethanol crude extracts of *Eurya japonica*. The extract showed good inhibitory activity on almost all the bacteria tested. The highest antibacterial activity of 13.33 mm were observed in *Klebseila pneumonia* on ethyl acetate extract and *Escherichia coli* on ethanol extract.The zone of inhibition in diameter (in mm) increases with increase in concentration of extract in disc. This showed the concentration dependent activity(3.3). Similarly among bacterial forms tested *E.coli* and *Pseudomonas* were found to be more sensitive to the acetone and ethanol crude extracts of *Ficus auriculata.* Other bacterial forms were inhibited by the exract(3.4). The highest antibacterial activity of 11.00 mm was observed in *Staphylococcus aures* on ethyl acetate extract.The inhibitory activities of the extracts live up to their potential in the treatment of microbial induced ailments or diseased conditions, in line with the traditional use of plant extracts. This investigation can be used in the folk medicine and source of antibacterial substances for possible treatment of many diseases including bacterial. However, to know the extract mechanism of action of *Eurya japonica* and *Ficus auriculata* leaf extract, further studies with purified fractions/ bioactive compounds are warranted .

REFERENCES

1. U.A. Stephen, F. Abiodun, O. Osahon and E. Ewaen, Phytochemical Analysis and Antibacterial Activity of *Khaya grandifoliola* Stem Bark, *Journal of Biological Sciences*, 2009; **9**: 63-67.
2. Fransworth, N.R., Morris, R.N. Higher plants: The sleeping giants of drug development. *American journal ofPharmaocology*, 1976; **17**(2): 46-56.
3. Web site used: http://www.ubcbotanicalgarden.org/education/eurya.php.
4. Facciola. S. Cornucopia - A Source Book of Edible Plants.Page No. 183.Oguyemi AO. In: Sofowora A. ed. Proceedings of a Conference on African Medicinal Plants. Ife-Ife: Univ Ife; 1979; 20-22.
5. Chi-Chih WU, Zhi-Fu HSU, and Chih-Hua TSOU,Phylogeny and taxonomy of *Eurya* (Ternstroemiaceae) from Taiwan, as inferred from ITS sequence data, *Botanical Studies* 2007; **48**: 97-116.
6. Web site used: http://senapati.nic.in/FloraandFauna.html.
7. Munesh Kumar and Vishwapati Bhatt Plant Biodiversity and Conservation of Forests in Foot Hills of Garhwal Himalaya, *A journal of ecology and application-lyonia*, 2006; **11**(2).
8. W. Roder, Rinzin and T. Gyeltshen,*Ficus auriculata* – its relative importance in Bhutan, farmers' preference and fodder quality, *Agroforestry Systems*, 2003; **57**: 11–17.
9. N.Sirisha, M.Sreenivasulu, K.Sangeeta,and C.Madhusudhana Chetty, Antioxidant Properties of Ficus Species –A Review, *International Journal of PharmTech Research*, 2010; **2**(4): 2174-2182.
10. Rizk, A.M., Constituents of plants growing in qatar: A chemical survey of sixty plants. *Fitoterapia*, 1982; **52**: 35-44.
11. Oguyemi AO. In: Sofowora A. ed. Proceedings of a Conference on African Medicinal Plants. Ife-Ife: Univ Ife; 1979: pp. 20-22.
12. Iyenger, M.A.,Study of Crude Drugs. 8 th edition Manipal power press. Manipal, India,1995; pp-2.
13. Somolenski, S.J, H. Silins and N.R. Farnsworth, Alkaloid screening I. *Lloydia*, 1972; **35**: 1-34.
14. Siddiqui, AA and Ali ,M., Practical Pharmaceutical Chemistry. 1st edition CBS publishers and Distributors, New Delhi, 1997; 126-131.
15. Ergene, A., Guler, P., Tan, S., Mirici, S., Hamzaoglu, E. and Duran, AAntimicrobial and antifungal activity of *Heracleum sphondylium* subsp. *artivinense*. *Afr.J. Biotechnology*,2006; **5**(11): 1087-1089.