JOURNAL OF INTERNATIONAL ACADEMIC RESEARCH FOR MULTIDISCIPLINARY
Impact Factor 1.625, ISSN: 2320-5083, Volume 3, Issue 2, March 2015

PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITY OF LEAF AND FRUIT EXTRACTS OF*HODGSONIA HETEROCLITA* (ROXB.) HOOK.f. &THOMSON

SILU BASUMATARY[1]
DAS, A. K[2]
RAAMAN, N[3]
SHARMA, G. D[4]
BAALAN. L[5]
BORA. R[6]

[1,2,6]Ethnobotany and Conservation of Medicinal Plants Laboratory, Department of Ecology and Environmental Science, Assam University, Silchar, India
[4]Bilaspur University, Bilaspur (Chattishgarh) India
[3,5]Laboratory of Natural Products, Centre for Advanced Studies in Botany, University of Madras, Guindy Campus, Chennai, Tamil Nadu, India

ABSTRACT

Hodgsonia heteroclita is a liane species belongs to the family Cucurbitaceae. A preliminary phytochemical screening of methanol extract of leaf and fruit of *Hodgsonia heteroclita* reveals the presence of alkaloids, carbohydrates, phenols, flavonoids, saponins, oils, terpenoids and steroids. The presence of these bioactive constituents was associated with the antimicrobial activity of the plant. The *in vitro* antimicrobial activity was studied by well diffusion method against human pathogens with two strains of gram positive and gram negative bacteria viz., *Escherichia coli, Staphyllococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis.* The gram negative bacteria appeared to be more susceptible than the gram positive bacteria. The results revealed significant inhibition activity of pathogens tested and the study supports that the leaf and fruit of *H.heteroclita* could be used as source of drug for bacterial infection. To the best of our knowledge, this may be the first report on antimicrobial activity that has not been previously reported and phytochemical screening is also observed for the first time from the extract of *H.heteroclita*.

KEYWORDS: Cucurbitaceae, *Hodgsonia heteroclita*, methanol extract, phytochemical screening, antimicrobial activity

INTRODUCTION

Plants, especially medicinal plants, offer vast resource of natural compounds with biological activities. Many medicinal plants have been screened extensively for their antimicrobial potential worldwide (Kaur and Arora, 2009; Mothana *et al*., Adedopo *et al*., 2009). Screening of various bioactive compounds from plants has lead to the discovery of new medicinal drug which have efficient protection and treatment roles against various

diseases (Kumar *et al.,* 2004; Mukherjee *et al*., 2007). Plant based medicines have been part of the traditional healthcare in most parts of the World for thousands of years (Chariandy *et al*.,1999, Newman *et al.*,2000).

Hodgsonia heteroclita (Roxb.) Hook.f. & Thomson is a liane fruit bearing plant of cucurbitaceae family. The family cucurbitaceae includes with 800 species and 130 genera (Jefferey, 2005; De Wilde and Duyfjes, 2006a, b, d) are among the economically important families and the commercial derivatives from medicinal species are increasing rapidly. The plant is collected from Kokrajhar district of Assam near the Bhutan border. The plant *Hodgsonia heteroclita* shows a wide distribution from southern temperate Asia (China; Guanxi, Xizang, Yunnan) to tropical Asia (de Wilde & Duyfjes 2001, GRIN 2007) and found to occur in India, Bangladesh, China, Malaysia and Nepal. In Northeast India it is distributed in hilly areas of Assam, Meghalaya, Arunachal Pradesh, Nagaland and Mizoram. It has only two species worldwide. It is a perennial climber reaching a size of upto 30m (de Wilde & Duyfjes, 2001). It has been used in the indigenous system of medicine for the treatment of various ailments. The leaves are also boiled and the resulting liquid taken internally both for nose complaints and to reduce fever (Hu, 1964). Seeds or fruit parts of some cucurbits are reported to possess purgatives, emetics and antihelmintics properties due to the secondary metabolite cucurbitacin content (Bisognin 2002; Rahman et *al*., 2008). In Northeast India, the fruit bulb is applied to bacterial infections in the feet (Changkija, 1999), intestinal worms (Semwal *et al*.,2014) and commonly used by bodo tribe against diabetes (Swargiary *et al*.,2013).Thus the present investigation was carried out to evaluate the antibacterial activity of *Hodgsonia heteroclita* with the leaves and fruits extract against several bacteria that can cause skin diseases and gastro-intestinal disorders in man.

MATERIALS AND METHODS

Collection of plant extract

The plant was collected in their flowering and fruiting stage. The plant parts collected are the fruits and the leaves. It was collected in the month of April and May and then it was identified and authenticated in BSI, Eastern Circle Shillong.

Preparation of plant extract

Fruits of the plant were collected, dried in shade and powdered. Soacked in methanol for 72 hours and shaken in Rotary Shaker, the extract was filtered using Whatmann no.1 filter paper.

To the extract, methanol was added again and the process continued till the colour of the filtrate was pale. All the filtrate were collected and concentrated in Rotary Vapor under reduce pressure and stored in 4^{o} C temperature for further analysis.

Phytochemical analysis

The condensed methanol extract of the leaves and fruits were used for preliminary screening of phytochemicals with the standard protocol described by (Evans, 1997; Kokate, 1999 and Raaman, 2006). The phytoconstituents tested were alkaloids, carbohydrates, glycosides, proteins, amino acids, saponins, phenols, flavonoids, terpenoids , phytosterols, fixed oils and fats.

Plant pathogenic bacterial cultures

The bacterial strains both gram positive (*Bacillus subtilis*- MTCC-441 and *Staphyllococcus aureus*- MTCC-96), gram negative (*Escherichia coli*-MTCC-1688 and *Pseudomonas aeruginosa*-MTCC-1687) were collected from Microbial Type Culture Collection (MTCC) and Gene Bank, Institute of Microbial Technology, Chandigarh, India were maintained at nutrient agar at refrigerated condition.

Anti-bacterial activity assay (Well diffusion method)

After culture inoculation of the MHA plates, sterilized 6mm cork borer was used to make agar wells and inoculums containing 10^{6} CFU/ml of bacteria were spread on the solid plates with a steril swab moistened with bacterial suspension. Then concentrations of 250µg, 500µg and 1000µg of the diluted samples were placed into each well, Kanamycin as a standard and 100% of DMSO as a control were used. The plates were incubated at 35-37^{o} C for 18-24 h. The diameter of the zone of inhibition was measured in mm around each well and the susceptibility was determined.

The percentage of inhibition zone was calculated by the formula=I (Diameter of the inhibition zone)/90 (Diameter of the petriplate in mm) x 100

RESULTS AND DISCUSSION

The preliminary photochemical screening for the methanol extract of *Hodgsonia heteroclita* leaf and fruit reveals the presence of medicinally bioactive constituents and showed positive for alkaloids, carbohydrates, saponins, terpenoids, oils, phenols and flavonoids. The

phytoconstituents like proteins, amino acids and glycosides were absence in leaf and fruit sample of *H. heteroclita*. Saponins, steroids and flavonoids were observed to be in high concentrations. (Mahmood *et al*., 2009) reported that phytochemicals such as alkaloids, saponins and terpenoids were potentially significant application against microorganisms.

The antimicrobial potential of leaf and fruit extracts (methanol extract) were evaluated by measuring the diameter of the zone of inhibition against the pathogens.The methanol extract showed effective against both gram negative bacteria and gram positive bacteria. In antibacterial studies, the methanol extract of leaf and fruit of *H.heteroclita* showed maximum inhibition against gram negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa* with sime diameter of 24mm and 22mm. Methanolic extract showed similar zone of inhibition against gram positive bacteria *Staphylococcus aureus* (22mm and 18mm) respectively followed by *Bacillus subtilis* (22 and 16mm) in leaf and fruit samples. The kanamycin used as control showed larger zone of inhibition for the test pathogens.

Table:1. Qualitative analysis of the phytochemicals in the leaf and fruit extract of *Hodgsonia heteroclita*

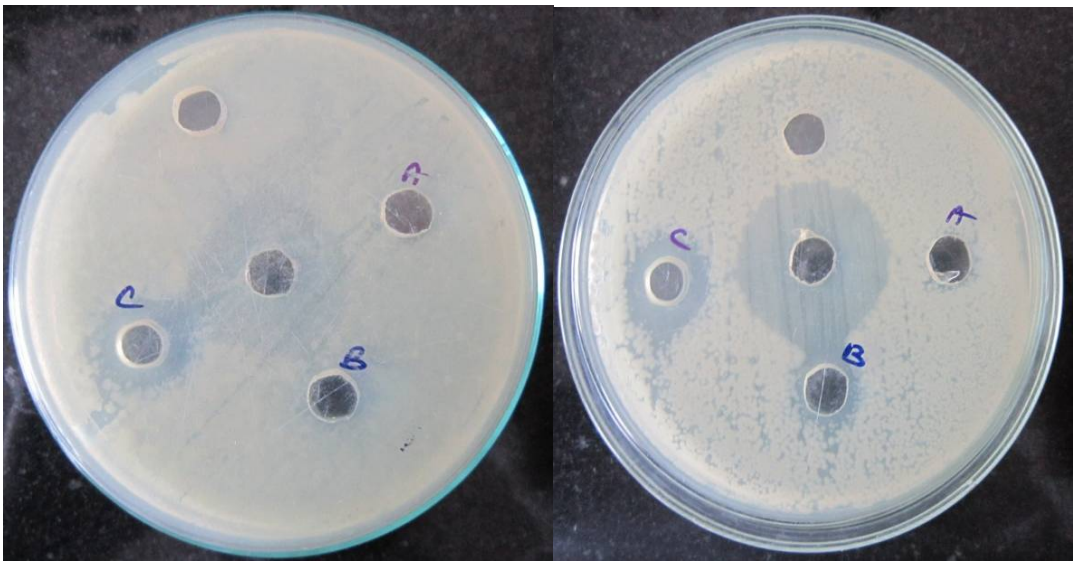
Types of Phytochemicals	**Name of the test**	**Methanol Extract**	
		Leaf	**Fruit**
Alkaloids	Mayer's test	_	_
	Wagner's test	+ +	+ +
Carbohydrates	Molisch's test	+ +	+ + +
	Fehling's test	_	+ + +
Glycosides	Borntrager's test	_	_
Proteins and Amino acids	Biuret reagent	_	_
	Ninhydrin	_	_
Saponins	Foam test	+ + +	+ + +
Phenolic compounds	Ferric chloride test	+ + +	+
	Gelatin test	+ +	+ + +
Flavonoids	Akaline test	+ + +	+ + +
Terpenoids	Salkowski test	_	+
Fixed oils and fats	Spot test	+ + +	+ +
Phytosterols	Libermann-Burchard's test	+ + +	+ + +

+ = presence of phytoconstituents; - = absence of phytoconstituents.

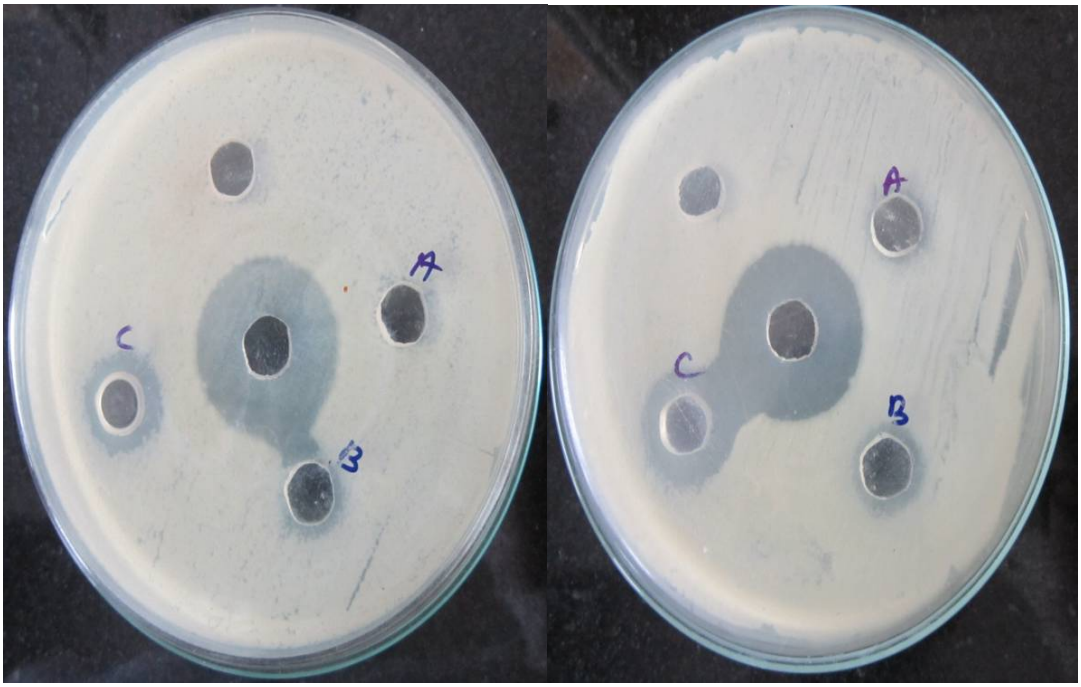
Table:2. Antibacterial activity of methanol extract of leaves and fruits of *Hodgsonia heteroclita.*

Plant part	Organism	Kanamycin	Zone of inhibition		
		30µg	250 µg	500 µg	1000 µg
Leaf	*Escherichia coli*	30	11.11	16.66	24.44
	Pseudomonas aeruginosa	30	14.44	15.55	24.44
	Bacillus subtilis	28	12.22	14.44	22.22
	Staphylococcus aureus	27	12.22	14.44	22.22
Fruit	*Escherichia coli*	30	13.33	15.55	22.22
	Pseudomonas aeruginosa	30	13.33	15.55	22.22
	Bacillus subtilis	25	12.22	13.33	16.66
	Staphylococcus aureus	25	12.22	13.33	18.88

Fig:1. Leaves and fruits of *Hodgsonia heteroclita* (Roxb.) Hook.f. &Thomson.

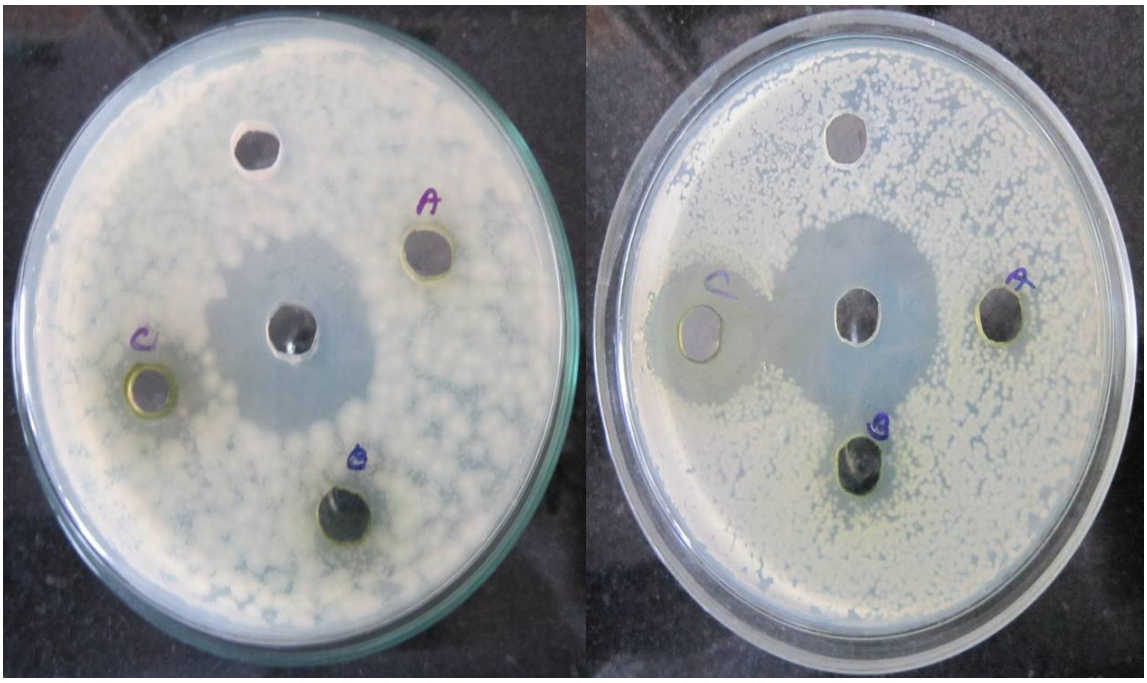


1.*Escherichia coli* 2. *Pseudomonas aeruginosa*

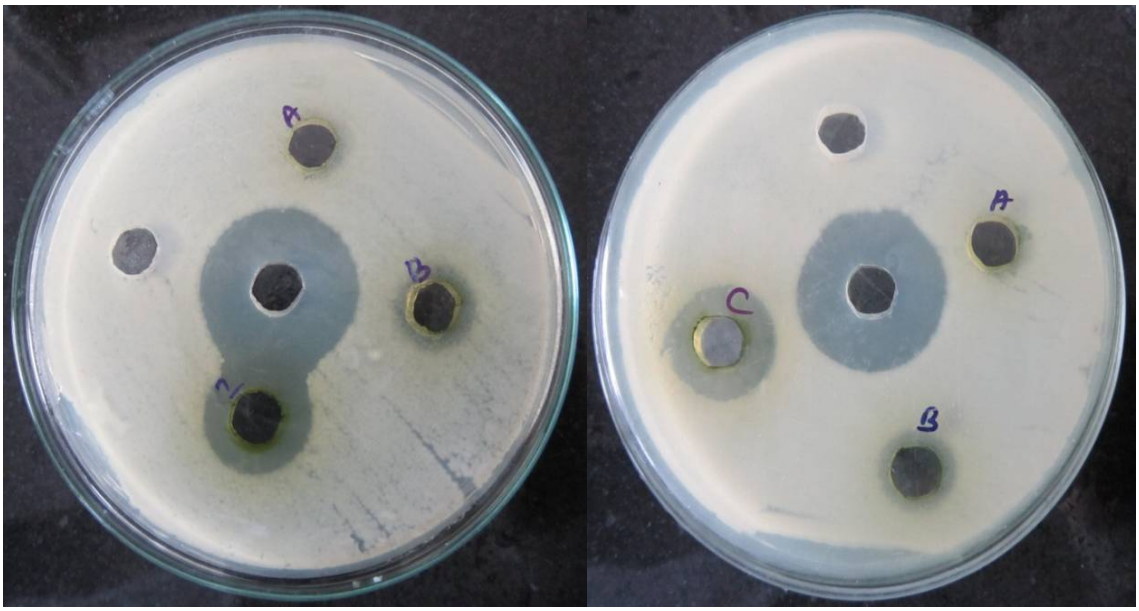


3. *Bacillus subtilis* 4. *Staphylococcus aureus*

Fig.2. Antibacterial activity of methanol extract of *Hodgsonia heteroclita* leaf (A). 250µg (B) 500 µg (C) 1000 µg concentrations.



A. *Escherichia coli* B. *Pseudomonas aeruginosa*



C. *Bacillus subtilis* D. *Staphylococcus aureus*

Fig.3. Antibacterial activity of methanol extract of *Hodgsonia heteroclita* fruit (A). 250µg (B) 500 µg (C) 1000 µg concentrations.

The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments (Nascimento *et al*., 2000; Rios and Recio, 2005). Phytochemical constituents in the plant samples are known to be biologically active compounds and they are responsible for different activities such as antioxidant, antimicrobial, antifungal and anticancer (Hossain and Nagooru, 2011; Suresh

and Nagarajan, 2009).The Knowledge of the phytochemicals is not only important for the discovery of healthcare products, but also in disclosing new sources of economic materials like tanins, oils, gums etc., (Farnsworth, 1966).The results of this study have shown that the plant contains phytoconstituents and antibacterial components. The methanol extract of *H.heteroclita* leaf and fruit posses significant inhibitory effect against the tested pathogens. The kanamycin used as control showed larger zone of inhibition for the test pathogens and this difference may be due to the fact that synthetic antibiotics are in pure form whereas crude extracts contain impure substances. In the present study, the increase in the diameter of the zone was may be due to the higher concentration of extracts i.e., 250µg, 500µg and 1000µg.This reveals that as the concentration of the extract increases, its antimicrobial activity also increases.

CONCLUSION

It is concluded that the results of present study reports that the different plant parts of *Hodgsonia heteroclita* have great potential as antimicrobial agents in the treatment of infectious diseases caused by resistance microorganisms. There is no previous report on evaluation of this plant concerning its antibacterial and phytoconstituent studies. The results support the traditional medicinal use of the plant. Further studies are going on this plant to isolate, identify and elucidate the structure of bioactive principles.

ACKNOWLEDGMENTS

I express my sincere gratitude to respected Prof. N. Raaman, Laboratory of Natural Products**,** Centre for Advanced Studies in Botany, University of Madras, Guindy Campus, Chennai, India for providing necessary facilities.

REFERENCES

1. Adedapo, A.A., Mogbojuri, O.M. and Emikpe, B.O. 2009. Safety evaluations of the aqueous extract of the leaves of *Moringa oleifera*. *Journal of Medicinal plants Research*. 3(8): 586-591.
2. Bisognin, D.A. 2002. Origin and evolution of cultivated cucurbits. Ciencia Rural, 32: 715-723.
3. Changkija, Sapu. 1999. Folk Medicinal Plants of Nagas in India. *Asian Folklore Studies*. 58: 219.
4. Chariandy, C.M., Seaforth, C.E. and Phelps, R.H. 1999. Screening of medicinal plants from Trinidal and Tobago for antimicrobial and insecticidal properties. *J Ethnopharmacol*. 64: 250-270.
5. De Wilde, W.J.J.O., Duyfjes, B.E.E., 2006a. Redefinition of Zehneria and four new related genera (Cucurbitaceae), with an enumeration of the Australasian and Pacific species. Blumea 51, 1–88.
6. De Wilde, W.J.J.O., Duyfjes, B.E.E., 2006b. Mukia Arn. (Cucurbitaceae) in Asia, in particular in Thailand. Thai Forest Bull. (Bot.) 34, 38–52.
7. De Wilde, W.J.J.O., Duyfjes, B.E.E., 2006d. *Scopellaria*, a new genus name in Cucurbitaceae. Blumea 51, 297–298.
8. El-Mahmood, A.M. 2009. Antibacterial activity of crude extracts of *Euphorbia hirta* against some bacteria associated with enteric infections. *Journal of Medicinal plants Res*. 3(7): 498-505.

9. Fransworth, N.R. 1966. Biological and Phytochemical screening of plants. *J Pharm Sci*, 35: 225-276.
10. GRIN 2007. *Hodgsonia heteroclita* information from NPGS/GRIN. Germplasm Resources Information Network (GRIN), United States Department of Agriculture.
11. Hossain, M.A. and Nagooru, M.R. 2001.Biochemical profiling and total flavonoids contents of leaves crude extract of endemic medicinal plant *Cordyline terminalis* L. Kunth. *Pharmacognosy Journal* 3 (24): 25-29.
12. Hu, Shui-Ying. 1964. The Economic Botany of *Hodgsonia*. *Economic Botany*. 18: 167-179.
13. Jeffrey, C., 2005. A new system of Cucurbitaceae. Bot. Zhurn. 90, 332–335.
14. Kaur, G. J. and Arora, D.S. 2009. Antibacterial and phytochemical screening of *Anethum graveoleus*, *Foeniculum vulgare* and *Trachyspermum ammi*. B.M.C Complement and Alternate Medicine. 9:30.
15. Kokate, C.K. 1999. Practical Pharmacognosy.4th edn. Vallabh Prakashan publication, New Delhi. p107-121
16. Kumar, R.A.,Sridevi, K., Kumar, N.V., Nanduri, S. and Rajagopal, S. 2004. Anticancer and immunostimulatory compounds from *Andrographis paniculata*. *J. Ethnopharma.*92: 291-295.
17. Mothana, R.A., Lindequist, U., Grunert, R. and Bednerski, P.J.2009. Studies of the in vitro anticancer, antimicrobial and antioxidant potentials of selected Yemini medicinal plant from island soqotra. BMC complement and Alternate Medicine.
18. Mukherjee, P.K., Kumar, V. and Houghton, P.J. 2007. Screening of Indian medicinal plants activity. *Phytotherapy Research*. 1142-1145.
19. Nascimento G.G.F., J. Locatelli, P.C. Freitas, G. L. Silva. 2000. Antibacterial activity of plant extracts and phytochemicals on antibiotic resistant bacteria. *Brazilian J. Microbial*. 31:247-256.
20. Newman, D. J., Cragg, G. M. and Snader, K. M. 2000. The influence of natural products
21. Raaman, N.2006. Phytochemical Techniques. New India Publishing Agency. Pitampura, New Delhi. 18-24.
22. Rahman, A.H.M.M., Anisuzzaman, M., Ahmed, F., Rafiul Islam, A.K.M and Naderuzzaman, A.T.M. 2008. Study of nutritive value and medicinal uses of cultivated cucurbits. *J. Applied Sci. Res., 4: 555-558.*
23. Rios J.L. and M.C. Recio. 2005. Medicinal plants and antimicrobial activity. *J. Thnopharmacol*. 100: 80-84.
24. Semwal, D.P.,Bhatt, K.C., Bhandari, D.C. and Panwar, N.S. 2014. A note on distribution, ethnobotany and economic potential of *Hodgsonia heteroclita* (Roxb.) Hook. F.& Thoms. In North-eastern India.
25. Suresh, S.N. and Nagarajan, N., 2009. Preliminary phytochemical and antimicrobial activity analysis of *Begonia malabarica* Lam. *Journal of Basic & Applied Biology* 3(1&2), 59-61.
26. Swargiary, A., Boro, H., Brahma, B.K. and Rahman, S. 2013. Ethno-Botanical Study of Anti-diabetic Medicinal plants used by Local people of Kokrajhar District of Bodoland Territorial Council. *Journal of medicinal Plant Studies.*1:51-58.
27. Trease G.E. and Evans, W.C. Textbook of Pharmacognosy. 14th edition. 24–28 Oval Road, London NW1 7DX, UK: W B Saunders Company Ltd.. pp. 13–53. Printed by Harcourt Brace & Company Asia Pte. Ltd. 583 Orchard Road No. 09-01 Forum Singapore 238884. 117–139, 293–334, 471-511
28. Upon drug discovery. Natural Product Reports. 17: 215-285.
29. Wilde, de W.J.J.O. & Duyfjes, B.E.E. 2001. Taxonomy of Hodgsonia (Cucurbitaceae) with a Note on the Ovules and seeds. Blumea 46: 169-179.