ejpmr, 2019,6(2), 367-373

SJIF Impact Factor 4.897

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

EXTRACTION, PHYTOCHEMICAL EVALUATION AND CHARACTERIZATION OF POLYPHENOLS AND FLAVONOIDS FROM AQUEOUS METHANOLIC LEAF EXTRACTS OF INDIGENOUS PLANT *CLERODENDRUM COLEBROOKIANUM* WALP AND *CENTELLA ASIATICA* LINN OF NORTH EAST INDIA

Ratna Jyoti Das*, Daphisha Marbaniang and Bhaskar Mazumder

Department of Pharmaceutical Sciences, Dibrugarh University.

***Corresponding Author: Ratna Jyoti Das**

Department of Pharmaceutical Sciences, Dibrugarh University.

Article Received on 10/12/2018 **Article Revised on 30/12/2018** **Article Accepted on 20/01/2019**

ABSTRACT

Extraction of polyphenols and flavanoids were done for two indigenous plants *Clerodendrum colebrookianum* walp and *Centella asiatica*. Extraction was done by both cold maceration and continuous hot percolation. Determination of total phenolic as well as and flavanoid content was carried out. Characterizations was done by TLC study, UV analysis, melting point determination and FTIR study. More total phenolic content found in *Clerodendrum colebrookianum* walp (621.02 ± 0.03 mg of GAE/gm plant extract) and content of total flavonoid was was found more in *Centella asiatica* L.(596.40 ± 0.53 mg of Quercetin equivalent/gm plant extract). UV and FTIR study concluded the existence of both flavonoids and phenolics in the respective extracts as compared with gallic acid and quercetin. Quantity of phenolic as well as flavanoid exhibit the indication of antioxidant action of the particular plants. Both the plants showing rich in total polyphenols and total flavonoids. Datas were mean ± SD moreover analyzed by means of One-way ANOVA, via Graphpad INSTAT. Confidence interval has been measured as 99% and $p< 0.01$ were considered significant. Phenolics encompass to possess an imperative antioxidant activity headed for free radicals, which is predominantly based on the redox properties of their phenolic hydroxyl groups and the structural associations among different parts of their chemical structure. It has been customary a highly positive rapport between total phenols along with antioxidant action in loads of plant species.

KEYWORDS: Polyphenols**,** flavonoids, extraction, indigenous plant, phytochemical evaluation.

INTRODUCTION

Medicinal herbs have a relevant and fundamental role to take part in attaining the goal of a proper human healthcare. The effective phyto-components obtained from plants are generally regarded as safe (GRAS) and are ecofriendly. The components used are believed to have better compatibility with human systems. Though effective, herbal medicines are not scientifically exploited, therefore this domain needs proper study in the light of modern science.[1] *Clerodendrun colebrookianum* Walp (Family, Verbenaceae) is individual of such imperative medicinal plants, widely used by the local natives of this region as a cardio protective agent and most universally well-known as Nefafu in Assam, Phuinum in Mizoram and Arun in Nagaland.[2,3] *Clerodendrum colebrookianum* is scattered extensively in the South as well as South-east Asia.[4] The Mizo community of North east expanse of India are claiming that low down frequency of hypertensive inhabitants among their population is due to the habitual intake of this remedial plant as vegetables. The plant exhibited to restrain triacontane triacontane, amyrin, clerodin, (24s) ethyl cholesta 5, 22, 25 trien 3-ol, clerodolone, clerodendoside, B-sitosterol, clerosterol, daucosterol, colebrin A-E.[5,6,7] The leaves along with leaf twigs of this plant are used for the home remedy of elevated blood pressure by the inhabitants of North-Eastern regions of India.[5,8] *Clerodendrum colebrookianum* Walp is suppose to be well thought-out as the most significant medicinal species which is used in the management of hypertension by different tribes of north east India.[8] However, the use of the plant for cure or treatment of diseases is based on administration of the leaves or flowers either by boiling or as vegetable.

Centella asiatica (L.) Urban, commonly called as Asian pennywort, Indian name guta kola and Thai name bua-bok, is a tiny creeping herb that has extensively been used in conventional medicine and a variety of purposes. It is a tropical healing plant with a lengthy history of remedial used for countless conditions as for instance dermal disease, vascular disorder, inflammatory and microangiopathy also.[9,10] Leaf extract of *Centella asiatica* exert persuasive antioxidative action as demonstrated by a range of assay system.[11] Leaf extracts of *Centella asiatica* (L.) possess prominently

elevated total phenolic content that is possess to contribute due to the presence of flavonoids as for instance quercetin, catechin, apigenin, rutin, naringin and kaempherol[12] and is directed to have a beneficial effect in suppressing blood pressure and is mostly considered to as a rejuvenate medicament mostly in Ayurvedic Pharmacopoeia.[13,14] A flavonoid biomarker quercetin which is found in the leaf extracts *Centella asiatica* (L.) was used as positive control in various study in view of the fact that it has been revealed to promote cardiovascular smooth muscle relaxation (antihypertensive effects) [15]

Aim of present study is to extract polyphenols and flavonoids from both the plants and phytochemical evaluation and characterization of the same.

MATERIALS AND METHODS

Collection and authentication of plant materials

The leaves of the plant *Clerodendrum colebrookianum* Walp. and *Centella asiatica* Linn. were collected from Sivasagar district of Assam, India during the month of June-July and January-February. Washing of the leaves were done thoroughly followed by shade drying and were preserved as herbarium sheet. The authentication was carried out in Botanical Survey of India (BSI), Eastern Regional Centre, Shillong, authentication number being DU/PSC/HRB/RJD/1/2016 and DU/PSC/HRB/RJD/2/2016 respectively.

Plant Extract Preparation

Grinding of the shade dried leaves to obtain powder passing through sieve no 60 of ASTM series. About 500 grams of the leaves powder were taken and extraction was done in a Soxhlet apparatus with aqueous methanol as a solvent. The extraction was allowed to continue for 72 hours and were concentrated to a dry mass by using a rotary evaporator (BUCHI, Switzerland) followed by lyophilization (IIC, India) into a dried mass by lyophilyzer. Cold maceration was also followed for extraction.

Phytochemical evaluation of plant extracts

Extracted material was subjected to various preliminary phytochemical screening to detect various secondary metabolites reported in Table 1. Detection of alkaloid (Mayer's test, Wagner's test, Hager's test, Dragendorff's test), carbohydrate (Molish's test, Fehling's test, Benedict's test, Barfoed's test), saponins, phenols (Ferric Chloride Test, Gelatin Test), glycosides (Borntrager's test, Lead Acetate Test), flavonoids(Magnesium and Hydrochloric acid reduction/Shinoda test, Alkaline reagent test), proteins and amino acids (Millon's test, Biuret test, Ninhydrin test, Legal's test, Keller-Killiani test), phytosterols (Liebermann- Burchard's test), fixed oils (Spot test, Saponification test), fats and gums and mucilages were performed.[16,17]

Chromatographic method

It is the method of partition of a mixture of components into single components. TLC study was performed to separate out individual components or secondary metabolites from the plant extract using different solvent systems.[16] The extracts was spotted on an activated pre-coated TLC plates (TLC Silica gel 60 F_{254}) and was exposed to various mixtures of organic solvents in different ratios for segregation of the constituents. R_f values were calculated applying the formula:

$$Rf = \frac{\text{Distance travelled by solute}}{\text{Distance traelleDd by solvent}} \quad \text{(Equation 1)}$$

Analytical methods used for characterization of the plant extracts

UV-Visible spectroscopy

Aqueous methanolic extracts of *C. colebrookianum* Walp and *C. asiatica* Linn, Quercetin and Gallic acid were examined by UV-Spectrophotometer in the range of 300nm to 800nm.

FTIR spectroscopy

Aqueous methanolic extracts of *C. colebrookianum* Walp and *C. asiatica* Linn, Quercetin and Gallic acid were determined by IR absorption spectrophotometer in the range 500 cm^{-1} to 4000 cm^{-1}.

Estimation of total phenolic content

The total phenolic content for the hydro methanolic extract of *C. colebrookianum* and *C.asiatica* were estimated using the method developed by Ordonez 2006 by applying Folin-Ciocaltau reagent with minor modifications.[18] The content of phenolics in extracts was expressed in Gallic acid equivalents (GAE). All samples were analyzed in triplicates.

Estimation of total flavonoids content

Content of the total flavonoid was calculated using the aluminum chloride colorimetric assay. The aqueous methanolic extracts of *C. colebrookianum* and *C.asiatica* were made solubilize in methanol in the concentration of 1mg/ml and diluted. 1ml of the diluted extract solution was taken in a test tube and 1 ml of 2% $AlCl_3$ solution dissolved in methanol. At room temperature all the samples to be incubated approximately for an hour. Determination of absorbance was done using UV VIS spectrophotometer (UV-1800 Shimadzu, Japan) at 415 nm. Calibration curve was prepared by using quercetin (standard compound) methanolic solutions at different concentrations between (10 – 90) µg/ml. The content of total flavonoids in extracts was demonstrated in terms of equivalent of Quercetin (QE).[19] All samples were analyzed in triplicates.

Statistical Analysis

All the datas were applied through statistical analysis. All values were expressed in terms of mean ± SD and analysis carried out by One-way ANOVA, using Graphpad INSTAT. Dunnet's multiple comparison tests was applied for the post-hock analysis to estimate

significance of difference among individual groups (**P<0.01). Confidence interval has been considered as 99% and $p< 0.01$ were considered significant. IC_{50} value was calculated by plotting a graph with percent inhibition on y-axis and concentration on x-axis.

RESULTS

Phytochemical evaluation of plant extracts

Specific reagents were used to perform the test, the results were observed either in the form of colour or precipitation reaction and additionally TLC profiling was done to suffice the specificity of the class of component. The results observed are given in Table 1, 2 and 3 and Figure 1 and 2.

Table 1: Phytochemical evaluation of plant extracts.

Sl. no	Constituents	*Centella asiatica*	*Clerodendrum colebrookianum*
1.	Alkaloid	+	+
2.	Carbohydrate	+	+
3.	Saponin	-	+
4.	Phenol compounds	+	+
5.	Glycosides	+	+
6.	Flavonoids	+	+
7.	Phytosterols	+	+
8	Protein and Amino acid	-	-

Table 2: R_f value and the solvent used as a mobile phase in *Clerodendrum colebrookianum*.

Constituents	Solvents used	Ratio	$R_{f\ value}$
Flavonoids	Chloroform – Acetone - Formic acid	(7.5:1.7:0.9)	0.85
	Ethylacetate - Chloroform	(4:6)	0.92
	Chloroform	(10)	0.81
	Benzene – Pyridine - Formic acid	(7.2:1.8:1.0)	0.93
	Ethylacetate - Formic acid - Glacial Acetic acid - Water	(6.8:0.7:0.7:1.8)	0.96
	Ethylacetate - Formic acid - Methanol-Water	(5:0.7:0.3:0.1)	0.82
Alkaloids	Toluene - Ethylacetate - Diethylamine	(7:2:1)	0.65
Anthracene Glycoside	Ethylacetate – Methanol – Water	(7.7:1.3:1)	0.73
Carbohydrates	Acetonitrile – Water	(8.5:1.5)	0.78
Saponin	Chloroform – Methanol	(9.9:0.1)	0.90
Protein	n-Butanol – Acetic acid – Water	(6:2:2)	-

Table 3: R_f value and the solvent used as a mobile phase in *Centella asiatica*.

Constituents	Solvents used	Ratio	$R_{f\ value}$
Alkaloids	Methanol: Ammonium hydroxide	17:3	0.71,0.78
Flavanoids	Chloroform: Methanol	18:2	0.16,0.30,0.45,0.60,0.73,0.82,0.85
Saponins	Chloroform:Glacial acetate:methanol:water	6:2:1:1	0.08
Terpenoids	Benzene: Ethyl acetate	1:1	0.36,0.40,0.46,0.73,0.86

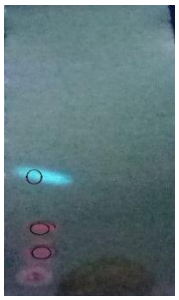

Fig. 1: TLC study of *C.asiatica.*

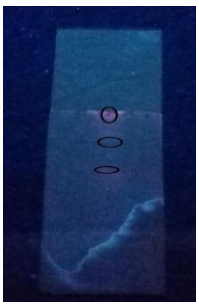

Fig. 2: TLC study of *C.colebrookianum*.

Analytical methods used for characterization of the plant extracts

UV-Visible spectroscopy

Aqueous methanolic extracts of *C. colebrookianum* Walp and *C. asiatica* Linn, Quercetin and Gallic acid were examined by UV-Spectrophotometer in the range of 300nm to 800nm and spectra are reported as Figure 3 to figure 6. Similar peaks were shown by the two extracts with standard flavonoids and phenols.

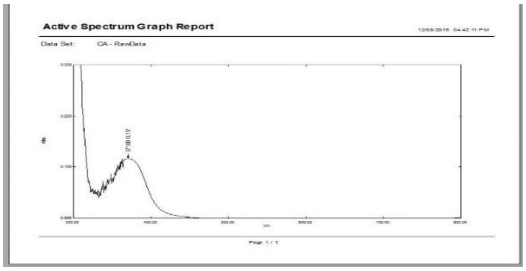

Fig. 3: UV spectrum of *C.colebrookianum*.

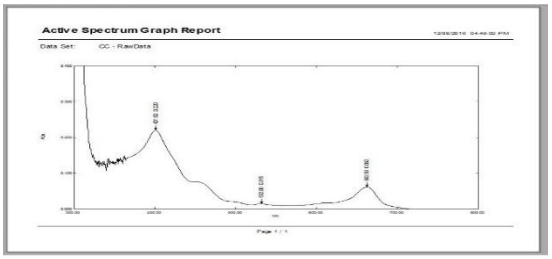

Fig. 4: UV spectrum of *C.asiatica*.

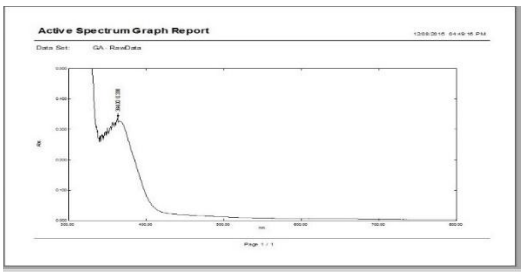

Fig. 5: UV spectrum of Gallic acids.

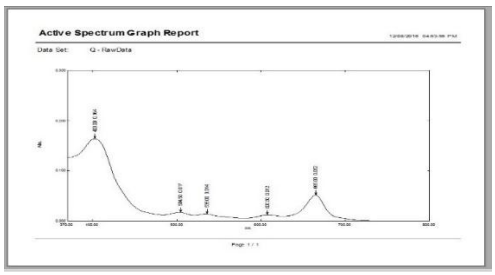

Fig. 6: UV spectrum of Quercetin.

FTIR spectroscopy

Aqueous methanolic extracts of *C. colebrookianum* Walp and *C. asiatica* Linn, Quercetin and Gallic acid were determined by IR absorption spectrophotometer in the range 500cm^{-1} to 4000cm^{-1} spectra are reported as Figure 7 to figure 10.

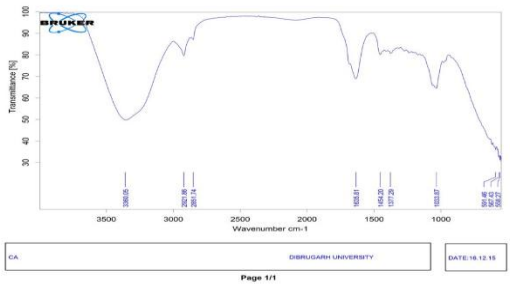

Fig. 7: FTIR Spectra of *C.asiatica*.

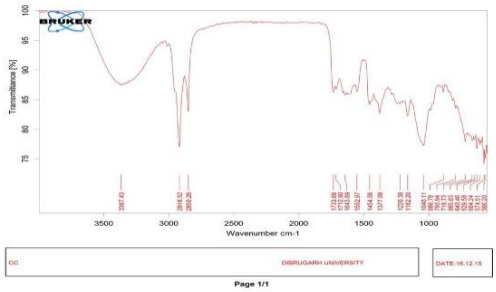

Fig. 8: FTIR Spectra of *C.colebrookianum*.

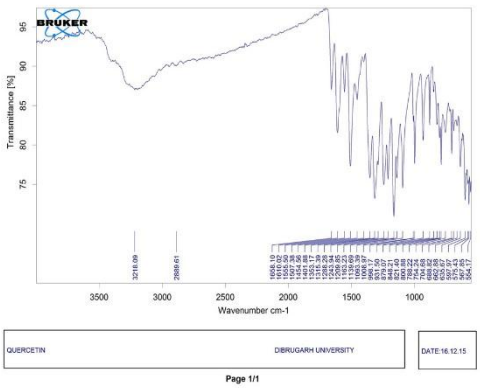

Fig. 9: FTIR Spectra of Quercetin.

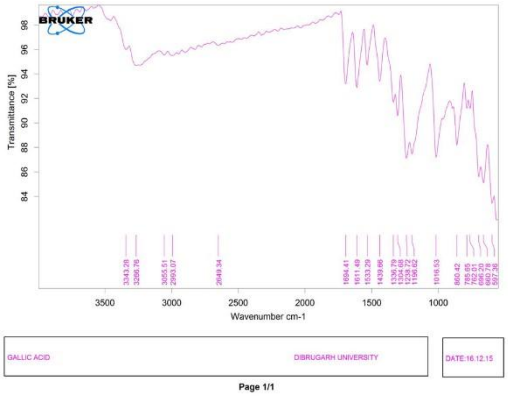

Fig. 10: FTIR Spectra of Gallic aids.

Interpretation of FTIR

From the FTIR study some groups were found common with standard flavonoids quercetin and standard phenol gallic acid. Illustrated in table 4.

Table 4: Interpretation of FTIR.

Functional group	Wave number cm^{-1}			
	Quercetin	Gallic acid	*Centella asiatica*	*C. colebrookianum*
Hydrogen bonded alcohol or phenol (OH)	3218.09	3343.28 3266.76 3055.51	3360.05	3367.43
CH_2 (Cycloalkane), hydrogen bonded acids	2889.61	2649.34	2921.86	2918.52 2850.26
C=O (ketone, aldehyde, ester, acid or amide)	1658.10	1694.41	-	1733.68 1712.90
Aromatic rings, alkene	1610.02 1555.50 1507.38	1611.49 1533.29	1635.81	1643.69 1552.97
CH (Alkane)	1454.56 1401.88	1439.66	2851.74 2921.86	1454.56 1377.09
C-O (Alcohols, Ethers)	1353.17 1288.28 1243.94 1209.85	1336.79 1304.68 1238.72	1377.29	1220.38

Estimation of Total phenolic content

The total phenolic content estimated to be enriched in the examined plant extract using the Folin-Ciocalteu's reagent is demonstrated in terms of GAE (gallic acid equivalent). The standard curve equation is y = 0.0115x, R^2 = 0.9907. The phenolic content for C. colebrookianum extract was found to be 621.02 ± 0.03 mg of GAE/gm plant extract whereas for C.asiatica was found to be 234.37 ± 0.54 and were shown in table 4.

Estimation of Total Flavonoid Content

Flavonoids are the most important and diverse phenolic compounds. The content of flavonoids present in the extracts was evaluated using quercetin as a standard. In the procedure the flavonoid present in the extract reacts with the aluminium ion (Al^{3+}) to form the stable flavonoid-Al^{3+} complex, which has a yellow color and whose intensity is proportional to the flavonoid concentration present in it. The content of flavonoids was expressed in terms of quercetin equivalent (the standard curve equation: y = 0.0191x, R^2 = 0.9956), mg of Quercetin/g of extract. The flavonoid content for *C. colebrookianum* extract was found to be 460.07 ± 0.11 mg of Quercetin equivalent/gm plant extract whereas for *C.asiatica* was found to be 596.40 ± 0.53 mg of Quercetin equivalent/gm plant extract and were reported in table 4.

Table 4: Total phenolic content and total flavonoid content.

Plant extract	Total flavonoid content	Total phenolic content
Clerodendrum colebrookianum Walp	460.07 ± 0.11	621.02 ± 0.03
Centella asiatica Linn	596.40 ± 0.53	234.37 ± 0.54

Values are expressed as Mean ± SD.

DISCUSSION

Phytochemical evaluation of plant extracts

Phytochemical test exposed the enrichments of constituents well known for vivid medicinal and physiological activities. The plant extracts are found to be enrich with polyphenols as well as flavanoids which are the main components for the anti-hypertensive activity although other classes of compounds also contribute for the blood pressure normalizing activity. From the knowledge of literature, the plants thus can be said to give good results when formulated in a dosage form.[24,25]

Thin Layer Chromatography

When flavonoids are exposed to UV radiation in a UV chamber (254-365nm) it is excited and known to produce yellow, green or blue fluorescent spots.[26] From R_f value data shown we can conveniently conclude that flavonoid is present, so are the other components showing their particular spots except protein.

Total phenolic and flavonoids content study

The conducted preliminary phytochemical studies confirmed the presence of flavonoids in test samples.[23] The total polyphenolic contents and total flavonoids enriched in the extracts were estimated in respect to standard gallic acid and quercetin respectively to understand the phyto– pharmacological relationship. The maximum total phenolic contents of *C.colebrookianum* was obtained as 621.02 ± 0.03 mg of GAE/gm plant extract whereas for *C.asiatica* was found to be 234.37 ± 0.54 mg of GAE/gm plant extract and total flavonoids enriched in *C. colebrookianum* extract was obtained 460.07 ± 0.11 mg of Quercetin equivalent/gm plant extract whereas for *C.asiatica* was obtained to be 596.40 ± 0.53 mg of Quercetin equivalent/gm plant extract. The

bioflavonoid bioflavonoid as well as biomarker quercetin has demonstrated for its antihypertensive property when administered chronically in the merely common rodent models of hypertension which may include high-sucrose diet induced hypertension[27] which reduces rise in blood pressure as well as heart rate. Moreover, free radicals causing damage towards G-protein coupling with receptors like muscarinic and a free radical scavenger, ascorbic acid offers prevention against reactive oxygen species at the receptor site. Hydroalcoholic Leaf extract of the indigenous plant *C. colebrookianum* and *C.asiatica* increases the antioxidant capacity of blood and had an inhibitory effect on the basal level of lipid peroxidation of liver and kidney[28,29], exhibited protection against ischemia–reperfusion heart injury in rat.[30] *C. colebrookianum* extract also enlightened the decrease in total cholesterol (TC) as well as low density lipoprotein (LDL) level and increased cardio protective high density lipoprotein (HDL) level.[29] The antioxidant action of both the extracts were found to be statistically significant when compared with control or standard. The extracts were found to have good antioxidant activity as evidenced from various radical scavenging activity results performed. Hence, it can be concluded that the plants can be used as good source of antioxidant which may be an important factor to efficiently lower the blood pressure, by reducing aldehyde conjugate/AGE formation resulting in reduced oxidative stress and enhancing resistance of insulin and other endothelial functions.[23,31-34]

CONCLUSION

The observations made in the present study revealed that both the plant *C.colebrookianum* and *C.asiatica* encompass considerable antioxidant action which might be supportive in preventing or lowering the evolution of various oxidative stress associated cardiovascular diseases.

➢ ACKNOWLEDGEMENT

- The authors are thankful to BSI, Shillong, Meghalaya, India for providing identification and authentication facility of the plant materials.
- The authors also extend their gratitude towards Division of Pharmaceutical Technology, Defence Research Laboratory, Tezpur, Assam, India to share their expertise.
- The authors are grateful to Dibrugarh University for providing laboratory facility and a Central Animal House Facility.
- The authors are grateful to University Grant Commission for funding the research work.

Funding

UGC, Rajiv Gandhi National Fellowship.

REFERENCES

1. Kamboj VP. Herbal medicine. *Current science*, 2000; 78(1): 35-51.
2. Nath, S.C. and D.N. Bordoloi., *Clerodendroncolebrookianum*a folk remedy for the treatment of hypertension in North Eastern India, Pharm. Biol., 1991; (29): 127-129.
3. Devi, R. and D.K. Sharma., Hypolipidemic effectof different extracts of *Clerodendroncolebrookianum* Walp in normal and high- fat diet fed rats, Journal of Ethnopharmacology, 2004; 90(1): 63-68.
4. Goswami P, Kotoky J, Chen ZN, Lu Y. A Sterol Glycoside from *C. Colebrookianum*, Walp' *Phytochemistry*, 1996; (41): 279–281.
5. Nath SC, Bordoloi DN, Clerodendroncolebrookianum, a folk remedy for the treatment of Hypertension in the North-eastern India. *Indian J Pharmacognosy*, 1991; (29): 127–129.
6. Bhuyan LR: Some plants used as medicines by the Nishi tribe of Arunachal Pradesh: A preliminary study. J. Econ. Taxon. Bot., 2003; 27(2): 447-450.
7. Buragohain J: Ethnomedicinal Plants Used by the ethnic Communities of Tinsukia District of Assam, India. Recent Res. Sci. Tech., 2011; 3(9): 31-42.
8. Nath SC, Bordoloi DN: Clerodendrumcolebrookianum, a Folk Remedy for the Treatment of Hypertension in Northeastern India. Pharmaceu. Biol., 1991; 29(2): 127-129.
9. M. T. De Sanctis, G. Belcaro, L. Incandela, M. R. Cesarone, M. Griffin, E. Ippolito, and M.Cacchio. Treatment of edema and increased capillary filtration in venous hypertension with total triterpenic fraction of *Centella asiatica*: a clinical, prospective, placebo controlled, randomized, dose-ranging trial. *Angiology*, 2001; 52(2): 55-59.
10. L. Incandela, G. Belcaro, M. R. Cesarone, M. T. De Sanctis, E. Nargi, P. Patricelli, and M. Bucci. Treatment of diabetic microangiopathy and edema with total triterpenic fraction of *Centella asiatica*: a prospective, placebocontrolled randomized study. *Angiology*,. 2001; (52): S27-S31.
11. L. Suguna, P. Sivakumar, and G. Chandrakasan. Effects of *Centella asiatica* extract on dermal wound healing in rats. *Indian Journal of Experimental Biology*, 1996; 34(12): 1208.
12. M. K. Zainol, A. Abd-Hamid, S. Yusof, and R. Muse. Antioxidative activity and total phenolic compounds of leaf, root and petiole of four accessions of *Centella asiatica* (L.) Urban. *Food Chemistry*, 2003; 81(4): 575-581.
13. M. K. M. Zainol. Determination of flavonoids in *Centella asiatica* (L.) Urban and their utilization in herbal noodles. Serdang, Malaysia: University Putra Malaysia, MSc Thesis, 2004.
14. D. M. A. Jayaweera. Medicinal Plants (Indigenous and Exotic) used in Ceylon, Part IV. *The National Science Council of Sri Lanka: Sri Lanka*., 1982; (55): 18.
15. M. Hussin, A. Abdul-Hamid, S. Mohamad, N. Saari, M. Ismail, and M. H. Bejo. Protective effect of *Centella asiatica* extract and powder on oxidative

stress in rats. *Food Chemistry*, 2007; 100(2): 535-541.
16. Kokate CK. *Practical Pharmacognosy*. 2005. 4th ed. New Delhi: Vallabh Prakashan.
17. Harborne JB. Phytochemical methods, A Guide To modern Technique of Plant Analysis. 1998; 3rd ed. London, UK: Chapman and Hall.
18. Ordonez AAL, Gomez JD, Vattuone MA, lsla MI. Antioxidant activities of *Sechium edule* (Jacq.) Swartz extracts. *Food Chemistry*, 2006; 97(3): 452–458.
19. Jain PK, Agrawal RK. Antioxidant and free radical scavenging properties of developed mono- and polyherbal formulations. *Asian J Exp Sci*., 2008; 22(3): 213-220.
20. Kokate CK. *Practical Pharmacognosy*. 2005. 4th ed. New Delhi: Vallabh Prakashan.
21. Mohamed AM. Prophylactic role of l-carnitine and ubiquinone in combating the cardio-toxicity induced by carbon tetrachloride in rat. *International journal of academic research*, 2010; 2(2): 52-59.
22. Montezano AC, Touyz RM.Molecular Mechanisms of Hypertension—Reactive Oxygen Species and Antioxidants: A Basic Science Update for the Clinician. *Canadian Journal of Cardiology*, 2012; 28: 288–295.
23. Deb L, Dutta A. Evaluation of mechanism for antihypertensive action of *Clerodendrum colebrookianum* used by folklore healers in North East India. *J Ethnopharmacol*, 2012; 143(1): 207-212.
24. Hodgson JM, Croft KD. Dietary flavonoids: effects on endothelial function and blood pressure. *J Sci Food Agri.*, 2006; 86: 2492–2498.
25. Edwards RL, Lyon T, Litwin SE, Rabovsky A, Symons JD, Jalili T.Quercetin Reduces Blood Pressure in Hypertensive Subjects. *J Nutr*., 2007; 137: 2405–2411.
26. Andersen OM, Markham KR. Flavonoids: Chemistry, *Biochemistry and Applications*. 2006; Boca Raton: CRC Press.
27. Francisco PV, Juan D, Rosario J, Celestino SB, Antonio O. Antihypertensive effects of the flavonoid quercetin. *Pharmacological Reports*, 2009; 61: 67–75.
28. Devi R, Banerjee SS, Maulik SK. In-vitro and in vivo antioxidant activity of different extracts of the leaves of *Clerodendrum colebrookianum* Walp in rat. *Journal of Pharmacy and Pharmacology*, 2003; 55: 1681-1686.
29. Devi R, Sharma DK. Hypolipidemic effect of different extracts of *Clerodendroncolebrookianum* Walp in normal and high- fat diet fed rats. *Journal of Ethnopharmacology*, 2004; 90(1): 63-68.
30. Devi R, Banerjee SK, Sood S, Dinda AK, Maulik SK.Extract from *Clerodendrum colebrookianum* Walp protects rat heart against oxidative stress induced by ischemic-reperfusion injury, *Life Sciences*, 2005; 77: 2999-3009.
31. Montezano AC, Touyz RM.Molecular Mechanisms of Hypertension—Reactive Oxygen Species and Antioxidants: A Basic Science Update for the Clinician. *Canadian Journal of Cardiology*, 2012; 28: 288–295.
32. Kizhakekuttu TJ, Widlansky ME. Natural Antioxidants and Hypertension: Promise and Challenges. *Cardiovasc Ther*., 2010; 28(4): e20–e32.
33. Vasdev S, Gill V. Antioxidants in the Treatment of Hypertension. *Int J Angiol*., 2005; 14(2): 60–73.
34. Ortiz MC, Manriquez MC, Romero JC, Juncos LA. Antioxidants Block Angiotensin II–Induced Increases in Blood Pressure and Endothelin. *Hypertension*, 2006; 38(2): 655-659.