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ANTIOXIDANT, MINERAL AND PHYTOCHEMICAL COMPOSITION OF *CLERODENDRUM COLEBROOKIANUM* WALP, A WELL KNOWN HOME REMEDY HERBAL FOR HUMANKIND

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ABSTRACT

Phytochemical and mineral constituents in crude extract of indigenous herbal *Clerodendrum colebrookianum* Walp**,** habitat wild belonging to *Verbenaceae*, has been explored. A number of novels bioactive organic compounds viz. antioxidant 60 µg/ml, saponin, 50 mg/g, flavonoid 20.641 mg/g ,alkaloid 25 mg/g, phenol 19.36 mg/g. tannin 18.374 mg/g, magnesium 5.31 mg/g, iron 0.38 mg/g, sulfur 1.35 mg/g, potassium 6.4 mg/g , manganese 0.66 mg/g ,calcium 10.3 mg/g, phosphorous 0.198 mg/g, zinc 0.13 mg/g, copper 0.06 mg/g, an indispensible sources of precious chemodiversity, the only means of survival forever on earth have depicted in the natures gifted region of north east India. The bioactive components not only add to supplements to food but also intensify the therapeutic chemicals for saving life from dreadful diseases, consequently reflect the sustainable development in hill plantation of the region.

Key words: *Clerodendrum colebrookianum*, bioactive constituents and minerals.

INTRODUCTION

Since the immemorial time of human civilization, the plants have been used by human beings for their health care needs. Trees and shrubs with medicinal and nutritional potentials which are indispensable constituents of human diets. They supply the body with minerals vitamins and certain hormone precursor in addition to proteins and energy . The medicinal value of plants are dictated by their phytochemicals and other chemical constituents (1).

Phytochemical have long been recognized to possess many properties including antioxidant, antiallergic, anti-inflammatory, antiviral antiproliferative and anticarcinogenic. (2). Clerodendrum is one of the high value wild habitat herbal having significance which is commonly practiced in Manipur. Further the genus *Clerodendrum* (*Verbenaceae)* is widely distributed in the tropics and subtropics , with a few species extending into the temperate regions. The species number has been estimated to be fivehundred and sixty (3) to fivehundred and eighty (4). The genus was first described by Linnaeus in 1757. The leaves are used as bitter tonic, vermifuge, laxative and cholagogue. Fresh leave juice is introduced in the rectum for the removal of ascarids and also believed to posses distinct antihelmintic properties. Leaves and roots are used for external applications on tumors (5).

MATERIAL AND METHODS

The plant *Clerodendrum colebrookianum* were collected from hill side S. Phunal, Chandel district Manipur on August - September 2010. The plant materials were identified by Botanical Survey of India (BSI) Shillong. The voucher specimen was deposited at HRDRI Canchipur. The plant material were cleaned, rinsed with demonized water and allowed to evaporate at room temperature and grind into uniform powdered in a grinder with almost care so as not to contaminate with dust.

Mineral element determination: The major elements comprising calcium, phosphorous, potassium, magnesium and trace elements (Iron, Zinc, copper, manganese and sulphur) were determined according to the method of analysis described by standard methods. (6-8).

0.2 g of the sample were weighed into a 50 ml flask, and 20 ml of nitric acid was added then the flask was placed on low hot plate in a digestion chamber. Then the flask is heated at higher temperature until the production of brown fume ceases. After cooling 10 ml of concentrated perchloric acid was added. This was heated vigorously until digestion was completed. The solution was allowed to cool and filtered using whatman No. 42 into a 100 ml volumetric flask and finally made upto mark with distilled water. The other minerals and chemicals were determined by using different methods viz. potassium by the flame photometer method, phosphorous by the vanado – molybdate yellow method and sulphur by spectrophotometer, calcium, iron, zinc, magnesium, copper and manganese by atomic absorption.

Determination of free radial scavenging of plant extracts by the use of DPPH radical: : 1 gm of the dried sample were weighed and put into 100 ml flasks. Each flask was added with 50 ml of 80% methanol. After one week of storage at room temperature the supernatants were filtered and these filtrate extracts were weighed. The extract sample was dissolved in methanol.(9).

DPPH radicals with maximum absorption of 515 nm compare to disappears due counter reaction of reduction by the antioxidant compound. The DPPH solution in methanol (6 X 10^{-5} M) was prepared daily, 3 ml of this solution was mixed with 100 µl of methanolic solution of plant extracts. The samples were incubated for 20 min at 37^{o}C in a water bath and then the decrease in absorbance at 515 nm was increased (AE). A blank sample containing 100 µl of methanol in DPPH solution was prepared (AB). These experiment was made triplicate for statistical error. Radical scavenging activity was calculated using the following formula

% inhibition = [(AB – AE) / AB] X 100.

Where,

AB = Absorbance of the blank sample

AE = absorbance of the plant extract

Determination of percentage of alkaloid and Saponin : The percentage of alkaloid and saponin were determined by the following method .(10-11).

Estimation of total flavonoid content : 0.1 gm of the dried sample with 20 ml of methanol were place at the room temp. for 48 hrs. The solvent was evaporated at room temp. and reduced upto 10 ml at room temp. After evaporation the sample were centrifuge at 10,000 rpm for 10 min. The supernatant were collected and volume was made upto 10 ml with methanol. 0.1 ml of supernatant with 0.1 ml of 10% aluminum chloride and 0.1 ml 1M potassium acetate and 2. 7 ml of distilled water to make volume upto 3 ml. After 30 minutes absorbance was measured at 415 nm. (12).

Total phenol determination : 0.2 gm of dried powder sample were extracted with 20 ml of 80% ethanol by using magnetic stir then it was centrifuge to homogenized at 10,000 rpm for 10 min. 5 ml of supernatant were dryness and dissolved in 5ml of distilled water. 0.1 ml of extract were volume made upto 3 ml with water and added 0.5 ml of Folin- Ciocalteu reagent

2 ml of 20% Na_2CO_3 solution shake well and place in a boiling water in one minute, cooled and measure the absorbance at 650 nm (13).

Tannin determination: 0.1 gm of powder sample with 10 ml distilled water were extracted for 3hrs. then it was centrifuge to homogenized at 10,000 rpm for 10 minutes. 0.1 ml of supernatant with 0.5 ml of Folin – Denis reagent 1ml of 35% Na_2CO_3 were volume make upto 10 ml of distilled water. Shake thoroughly and read the absorbance at 700nm after 30 minutes (14).

RESULTAS AND DISCUSSION

Investigation on phytochemical constituents of *Clerodendrum colebrookianum* yields a number of organic compounds inclusive of antioxidant and shown in Table 1. The potential of concentration of antioxidant ranged to 60 µg/ml, saponin 50mg/g, flavonoid 20.6 41mg/g , alkaloid 25 mg/g, phenol 19.36 mg/g and tannin 18.374 mg/g. Graphically it is represented in fig. 1 and fig. 3.

The mineral composition of the test herbal is shown in Table-2 and fig.2. Among the test minerals, calcium account 10.3 mg/g, in top followed by potassium 6.4 mg/g, magnesium 5.31 mg/g, phosphorous 0.198 mg/g, sulfur 1.359 mg/g, manganese 0.66 mg/g, iron 0.38 mg/g, zinc 0.13mg/g, copper 0.06 mg/g and cobalt nil.

Table 1. The phytochemical constituents of flavonoid, saponin, alkaloid, phenol, tannin in mg/g and antioxidant in µg/ml of the Clerodendrum colebrookianum.

Plant species	Antioxidant IC_{50} (µg/ml)	Flavonoids	Saponins	Alkaloids	phenol	Tannin
Clerodendrum colebrookianum.	60	20.641	50	25	19.36	18.374

The values are mean of three observations.

Table 2. Composition of Mineral elements of *Clerodendrum colebrookianum* in mg/g

Plant Parts										
	K	**Mg**	**P**	**Ca**	**S**	**Fe**	**Zn**	**Mn**	**Cu**	**Co**
Leaves	6.4	5.31	0.198	10.3	1.359	0.38	0.13	0.66	0.06	ND*

*ND= Not detected.

The values are mean of three observations.

Perusal on Table-1 of present investigation revealed that the fully matured plant of *Clerodendrum colebrookianum* were free resources of therapeutic organic compounds of antioxidant, phenol, saponin, flavonoid, alkaloid and tannin that available wild in nature. The presence of these bioactive compounds have empathetically emphasize the medicinal potentials of the test herbal similar result have reported from different plants and countries. (15-16).

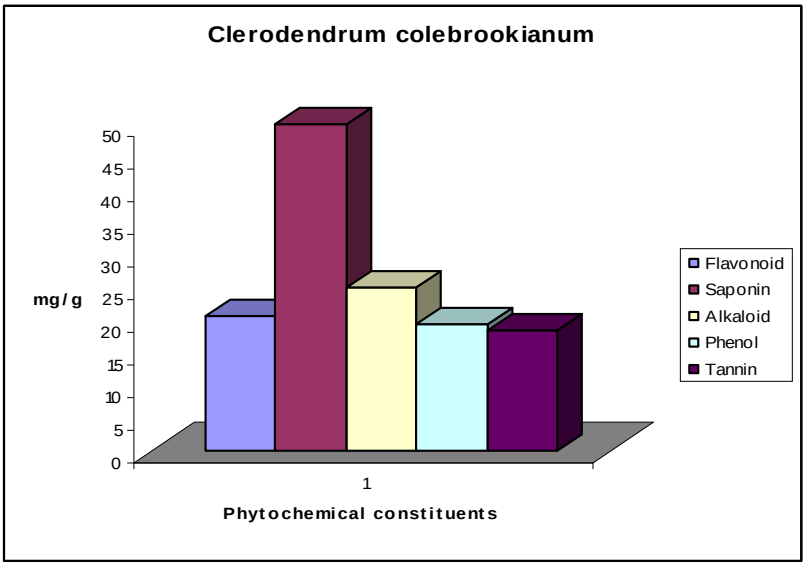

Fig. 1. Phytochemical composition of *Clerodendrum colebrookianum* .

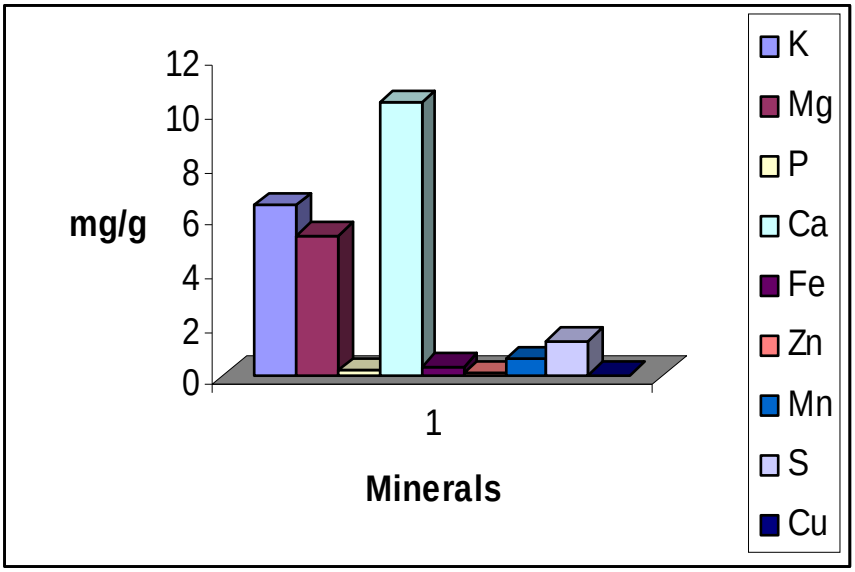

Fig. 2. Mineral composition of *Clerodendrum colebrookianum* .

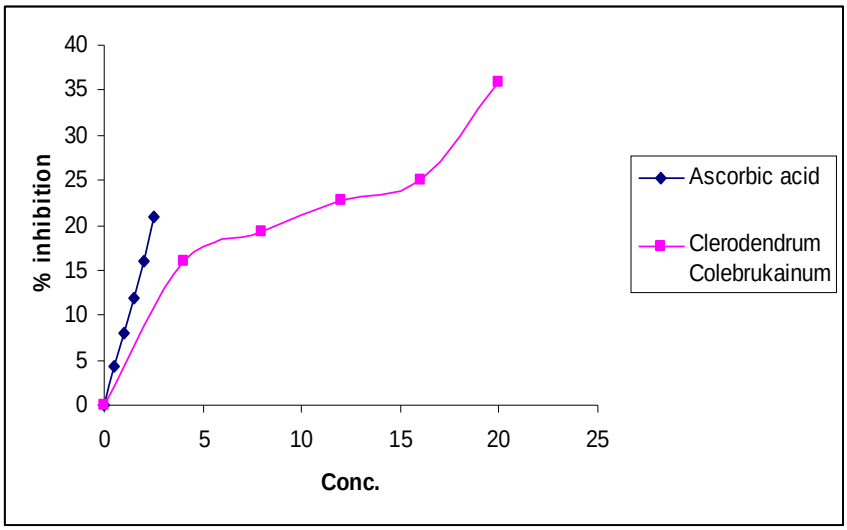

Fig. 3.DPPH free radical scavenging activity of methanolic extract *Clerodendrum colebrookianum* added to methanolic solution of DPPH as compared to Ascorbic acid.

The present phytochemical test of plant extract depict an antioxidant content upto 60 µg/ml under free radical scavenging activity technique confirmed the unique presence of the compound in *Clerodendrum colebrookianum*. Antioxidant and its ability to balance or trap highly reactive free radicals and oxygen species are very essential in biological systems from a wide variety of sources for longer life of cells. These free radicals may oxidised nucleic acids, protein, lipids or DNA and can initiate degenerative diseases. Further antioxidant compounds like phenolic acid, polyphenols and flavonoid scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanism which leads to degenerative disease. There are number of clinical studies suggesting that the antioxidant in fruits, vegetables, tea and red wine are the main factors for the observed efficacy of these foods in reducing the incidence of chronic diseases including heart disease and some cancer (17).

Table 1. delineated the phenolic compounds content upto 19.36 mg/g in the leaves of *Clerodendrum colebrookianum*. Phenol is readily absorbed by all routs, and rapidly distributes throughout the body. Following dermal or inhalation exposure, the half- life of phenol in the human body is approximately 3.5 hours. Unchanged phenol and its metabolic products are primarily excreted in the urine. Phenols have been subjects of extensive research as disease preventives. (18-19). Phenols have been responsible in having the ability to block specific enzymes that causes inflammation. They also modify the prostaglandin pathway and thereby protect platelets from clumping.

Table 1. depict that the content of alkaloid ranged to 25 mg/g among the phytoorganic compounds . The plant parts uses in the treatment of various diseases may be due to presence of traces of alkaloids. Alkaloids in Euphobia species used as purgative (20). Similarly *Momordica charanta* and *Azadirchta indica* used in the cure of malaria also contain alkaloid (21-22).

Close examination on the present investigation accounts flavonoid upto 20.641 mg/g with other phytochemical compounds (table 1). Flavonoids generally represent the most common and widely distributed groups of plant phenolics. Flavonoids are potent water- soluble super antioxidant and free radical scavengers which prevent oxidative cell damage, have strong anti- cancer activity and protect against all stages of carcinogenesis (23-24). Flavonoids were reported to possess biologically activity against microbes (25).

The present phytochemical test determined the presence of saponin with 25 mg/g (Table1). Saponin are consciously a common focus in plant research. Saponin generally used freely as an expectorant, secretolytic and antispasmodic in response to specifically, whooping cough, spastic, bronchitis, and chronic catarrh (26). Emollient and itchrelieving preparations, including creams, lotions, and shampoos, are used cosmetically and in the treatment of skin disorders (27).

The present investigation determined the tannin content with 17.03 mg/g (Table 1) of the test herbal. Tannin is believed to be the principal active substance of several plants used in folk medicine. Most of the Ayurveda and Siddha tooth powders contain tannin-bearing materials to strenghthen the gums. Tannin is the praparative of gallic acid. Gallic acid is used as key intermediate in the manufacture of an antifolic, antibacterial drug “Trimethoprim”. (28). A similar trend was reported earlier from therapeutic plant (29).

Minerals are naturally occurring chemical elements found throughout the human body in the bones, muscles, teeth, blood and nerve cells. Minerals help to maintain a normal water balance within the body, stimulate a healthy immune system (iron), fighting cancer cells (manganese), antibiotic capabilities (sulfur), helping wounds heal, production of white blood cells (zinc). Magnesium can help to prevent insulin resistance, that is, the inability to use insulin efficiently to turn glucose into energy. As a result of insulin resistance, glucose levels rise, which can lead to diabetes and heart disease. Calcium alone is insufficient to maintain a healthy body; needed magnesium and potassium to assist calcium in its essential functions.

These three nutrients work together to contact and relax muscles, maintain a healthy nervous system. (30).

CONCLUSION

The present investigation vividly revealed that the *Clerodendrum colebrookianum* have unique medicinal value with high phytochemicals and minerals which can be utilized in the treatment of numerous dreadful diseases and also be explore for using in consumption in pharmaceutical, cosmetic industries and chemo diversity conservation. Eventually make a proposition for prestigious *Clerodendrum colebrookianum* for hill plantation in the region for sustainable development, the way of right utilization of resources without compromising to future users.

Furthermore, consequent to the high phytochemicals and mineral contents, the *Clerodendrum colebrookianum* may be used as compulsive resource of potential sources for useful food in daily diet and needful drugs. Studies in depth are highly needed for fully exploration on plantation, harvest, postharvest and storage of products for enhancement of standardization of life in the region.

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REFERENCES

1. Fallah, H.S.M.; Alavian, H.R. Heydari, M.R. Abolmaali, K. The efficacy of Liv- 52 on liver cirrhotic patients: a randomized double blind, placebo- controlled first approach. Phytomedicine 2005;12(9): 619-624.
2. Eastwood, M.A. Quarterly J. Med. 1999; 92,527.
3. Moldenke, H.N. A fifth summery of verbenaceae nymphoremaceae and eriocaneacae of the world as to valid taxa geograpic distribution synonumy vol. 1. Braun- Brumfielt, Inc. Ann. Abor, Michigan, 1971; 312-314.
4. Munir, A.A. A taxonomic revision of the genus Clerodendrum L. Verbenaceae in australia J. Adelaide Bot. Gard, . 1989 ; 11(2): 101-173.
5. http://www.aturemagics.com/ayuveda.2010.
6. Capar, S.G.; Tanner, J.T. Friedman, M.H. and Boyer, K.W. Multielement analysis of animal wast and sewaze. Studge Environ. Sci. Technol. 1978;12. 785.

7. Gupta, P.K. Soil, Plant, Water and Fertilizer analysis. Agrobios (India) Agro House, Behind Nasrani Cinema Chopasani Road, Jodhpur, 2006.; 438.
8. Tandon H.L.S. Method of analysis of soils, plants, waters and Fertilizers. Fertilizer development and consultation organisation 204-402A Bhanot corner 1-2 pamposh Enclave. New Delhi 1993; 143.
9. Dudonne, S. Vitrax, X. Coutricre, p. Woilez, M. & Merillon, J. comparative stuy of antioxidant properties and total phenolic content at 30 plants extract at industrial Interest using DPPH, ABTs, FRAP, DOS and ORAC Assay J. Agrc. Chem. 2009; 57: 1768-1774.
10. Hamid, R.M; Ali, G. & Mehrdad, I. Antinociceptive effects of Peganum harmala, L. alkaloid extract of mouse formaline test. Canadian society of pharmaceutical science. 2004.
11. Obadoni, B.O. and Ochuko, P.O. Phytochemical studies and comparative efficacy of crude extracts of some homeostatic plants in Edo and Delta states of Nigeria. Global J. Pure and Applied Science , 2001; 8: 203-208.
12 . Chang, C. Yang, M. Wen, H. Cherr, J. Estimation of total flavonoid content in protein by two complementory coloremetric method. J. Food Drug Analy 2002.;10: 178 – 182.
13. Malick, C.P. and Singh, M.B.. in: Plant Enzymology and Histoenzymology, Kalyani publication, New delhi 1980; 286.
14. Sadasiyam, S. and Manickan, A. In: Biochemical method for Agricultural science, Wiley Eastern Ltd. New Delhi , 1992; 189-191.
15. Blumgeter, P. Textbook of matezrial medicine pharmacologzy and the king Saud press London, 1989;7. 11-13.
16. Abdulrahaman, F.A. Onyeyili, P.A. Phytochemical srening and pharmacological activities of the stem Bark of Terminalia avicennoids. Bull. Anim. Hlth. Prod. Afr. 2001;49: 236-242.
17. Miller , H.E. Rigelhof, F. Marquart, L. Prakash, A and Kanter, M Cereal foods world.2000; 45(2), 59-63.
18. Okwu, D.E. and Omodamiro, O.D. Effects of hexane extract and phytochemical content of Xylopia acthiopica and Ocimum gratissimum on the uterus of Guinea pig. Bio. Res., 2005; 3.
19. Duke, J. Handbook of biological active phytochemicals and their activities. BOCA Raton (FL) CRC Press, 1992.; 99-131.
20. Ralula, T.N.; Rukhsndra, A.; Zaidi,A. and Shamshila, phytochemical screening of medicinal plants belonging to the family Euphorbiaccal. Pak. Vert, J. 1994;1: 160-162.

21. Harbone, J.B. phytochemical methods. Chapman and Hall Ltd. London 1973; 11-113.
22. Haslam, E. Plant Polyphenols, vegetable tannins revisited. Cambridge University Press, Cambridge, 1998; 250.
23. Salah, W.; Miller, N.J.; Paguaga, G.; Tijburg, G.P. Bowel, E. Rice and Evans, C. poluphenolic flevonols as scavenger of aqueous phase radicals as einbreaking antioxidants. Arch. Biochem. Biorh., 1995;.2: 339-346.
24. Okwu, D.E. phytochemical and vitamin content of indigeneous species of south eastern Nigeria J. Sustain Agric. Environ, 2004; 6: 30-34.
25. Kensella, J.E. Food Technology 1993; 47: 85-90.
26. Wichtl, M and Bisset, N.G.. Herbal drugs and phytopharmaceuticals. Stuttgart: Medpharm Scientific Publisher. 1994.
27. Bruneton, J. Pharmacognosy, phytochemistry, medicinal Plant. Paris: Livoisier Publishing, 1995.
28. Ramakrishnan, K. Selvi, S.R. and Shubha, R. Tannins and its analytical technique. Indian Chemical Engr. Section 2006; A. Vol. 48, No.2.
29. Singh, S.R. Devi, M.N. and Devi, N.S. Antimicrobial activity and phytochemical constituents of fruit and seed extract of Xanthoxylum rhetsa (Roxb) DC. Env. & Ecoplanning. 2010;Vol 17.No.1.
30. www.iny.rose.co.uk/Human Body-Science/Mineral.2010.