QUALITATIVE PHYTOCHEMICAL ANALYSIS OF SOME LEAFY VEGETABLES USED TO CURE ANEMIA IN WEST SINGHBHUM, JHARKHAND, INDIA

Dolly Tudu¹ and Vishnu Shankar Sinha²

¹Department of Botany, Kolhan University Chaibasa, West Singhbhum, Jharkhand – 833202
²Department of Botany, Tata College, Chaibasa, West Singhbhum, Jharkhand – 833202

ARTICLE INFO

Article History:
Received 18th February, 2017
Received in revised form 10th March, 2017
Accepted 06th April, 2017
Published online 28th May, 2017

Key Words:
Ethnomedicine, Phytochemical, Qualitative, Secondary metabolites.

ABSTRACT

Present study reports the preliminary qualitative phytochemical analysis of six medicinal plants viz., Amaranthus tricolor L. (Amaranthaceae), Centella asiatica L. (Apliaceae), Chenopodium album L. (Amaranthaceae), Cicer arietinum L. (Fabaceae), Colocasia esculentum L. (Araeaceae) and Ipomia aquatica L. (Convolvulaceae). The qualitative phytochemical analysis was carried out after extracting sample using soxhlet extraction by using double distilled water, ethanol and methanol as extractants. Various tests were carried out for the detection of phytochemicals such as Alkaloids, Flavonoids, Saponins, Phenols, Carbohydrates, Tannins, Protein and Glycosides in the plant extracts and iron spot was tested by 5% potassium thyocinate solution (KSCN). Our result confirmed the presence of major classes of phytochemical and iron in the plant leaves extracts. This preliminary study draws attention to the need for further studies to know the exact active secondary metabolites which is responsible for the treatment of Anemia and other diseases.

INTRODUCTION

Over the years, medicinal plants have been recognized to be of great importance to the health of individuals and communities; used as traditional medicines and are continuously providing new remedies to mankind (Alimmoladun et. al., 2007). In pregnant and nursing mothers plants leaves are used for medicinal and nutritional purposes (Okwu, D.E.; 1999; Okwu, D.E.; 2001). Kattimani et. al., (2000) reported that over 75% of the world population is still depending on local health practioner and traditional medicines for their primary health care. Traditional knowledge provides clues to the discovery of valuable drugs (Buenz et. al.; 2004).

Plant based foods are source of both energy and nutrition which is essential to the health (Ughade et.al.,1998; Belanger et.al.,2004) and phytochemicals of the plants produce a definite physiological action on the human body. Green leafy vegetables are the cheapest source of the food with richest nutritional value within the reach of poor people (Kuhnlein H.V. and Receveur O.; 1996).

Green leafy vegetables are rich source of vitamins, minerals (Zinc, Iron, Potassium etc) and also contains bioactive phytochemicals which provides several health benefits i.e., protection from cardiovascular diseases (Okeno and Chebert; 2003), anti-inflammatory, anti carcinogenic, antimalarial, inhibition of cholesterol synthesis, antiviral, antifungal and antibacterial activity (Mahato and Sen;1997), antidiabetic, hepatopoeotective, antioxidant (Ruckmani et al 1998).

Anemia is a widespread public health problem associated with an increased risk of morbidity and mortality, especially in pregnant women and young children. It is a condition caused due to both nutritional (vitamin and mineral deficiencies) and non nutritional factors or due to deficiency of Iron (Madukwe, et.al.; 2013). The vulnerable groups of iron deficient are infants, young children and women of child-bearing age (WHO, 1968).

Iron bioavailability is influenced by the degree of iron deficiency of the individual, the adequacy of intestinal secretions, and the various components of food that inhibit or enhance iron absorption. Most cases of iron deficiency are mild and do not results in symptoms that are recognized as requiring medical attention (Oladiji, 2003).

Present paper reports the phytochemical analysis of six plants of five familes which is being used as green leafy vegetables to defeat anaemia by tribal and rural people of West Singhbhum, Jharkhand, India.

*Corresponding author: Dolly Tudu
Department of Botany, Kolhan University Chaibasa, West Singhbhum, Jharkhand – 833202
MATERIAL AND METHOD

Collection and Processing of Plant Materials: The fresh plant leaves were collected from the nearby villages and identified with the help of many references books like “Indian Medicinal Plants” (Kritikar and Basu, 2012), “Glossary of Indian Medicinal Plants with active principle Part I (A-K)” (Asolkar, et al., 1996), “Glossary of Indian Medicinal Plants” (Chopra, et al., 1996). The plant materials were air-dried in the laboratory for two weeks and then ground into powdered by using a mortar and pestle and powders were stored into an airtight container with proper labeling for future use.

Preparation of plant extracts: Crude plant extract was prepared by soxhlet extraction method and 20 g of powdered plant material was uniformly packed into a thimble and extracted in 250 ml of different solvents i.e., distilled water, methanol and ethanol separately. The process of extraction continues for 24 hours or till the solvent in siphon tube of an extractor become colorless. After that extract was taken in a beaker and kept on hot plate and heated at 30-40°C till all the solvent got evaporated. Dried extract was kept in refrigerator at 4-6°C for their future use in phytochemical analysis.

Qualitative Phytochemical analysis: The extract was tested for the presence of bioactive compounds by using following standard methods (Sofowara, 1993, Trease and Evans, 1998, Harborne, 1973).

Test for proteins

Millon’s test
Crude extract when mixed with 2ml of Millon’s reagent, white precipitate appeared which turned red upon gentle heating that confirmed the presence of protein.

Ninhydrin test
Crude extract when boiled with 2ml of 0.2% solution of Ninhydrin, violet colour appeared suggesting the presence of amino acids and proteins.

Test for carbohydrates

Fehling’s test
Equal volume of Fehling A and Fehling B reagents were mixed together and 2ml of it was added to crude extract and gently boiled. A brick red precipitate appeared at the bottom of the test tube indicated the presence of reducing sugars.

Benedict’s test
Crude extract when mixed with 2ml of Benedict’s reagent and boiled, a reddish brown precipitate formed which indicated the presence of carbohydrates.

Iodine test
Crude extract was mixed with 2ml of iodine solution. A dark blue or purple coloration indicated the presence of the carbohydrate.

Test for phenols and tannins
Crude extract was mixed with 2ml of 2% solution of FeCl3. A blue-green or black coloration indicated the presence of phenols and tannins.

Test for flavonoids
Shinoda test Crude extract was mixed with few fragments of magnesium ribbon and concentrated HCl was added drop wise. Pink scarlet colour appeared after few minutes which indicated the presence of flavonoids.

Alkaline reagent test
Crude extract was mixed with 2ml of 2% solution of NaOH. An intense yellow colour was formed which turned colourless on addition of few drops of dilute acid which indicated the presence of flavonoids.

Test for Saponins
Crude extract was mixed with 5ml of distilled water in a test tube and it was shaken vigorously, the formation of stable foam was taken as an indication for the presence of Saponins.

Test for glycosides

Liebermann’s test
Crude extract was mixed with each of 2ml of chloroform and 2ml of acetic acid. The mixture was cooled in ice. Carefully concentrated H2SO4 was added. A colour change from violet to blue to green indicated the presence of steroidal nucleus, i.e., glycone portion of glycoside.

Salkowski’s test
Crude extract was mixed with 2ml of chloroform. Then 2ml of concentrated H2SO4 was added carefully and shaken gently. A reddish brown colour indicated the presence of steroidal ring, i.e., glycine portion of the glycoside.

Keller-kilani test
Crude extract was SI mixed with 2ml of glacial acetic acid containing 1-2 drops of 2% solution of FeCl3. The mixture was then poured into another test tube containing 2ml of concentrated H2SO4. A brown ring at the interphase indicated the presence of cardiac glycosides.

Test for steroid
Crude extract was mixed with 2ml of chloroform and concentrated H2SO4 was added sidewise. A red colour produced in the lower chloroform layer indicated the presence of steroids.

Another test was performed by mixing crude extract with 2ml of chloroform. Then 2ml of each of concentrated H2SO4 and acetic acid were poured into the mixture. The development of a greenish coloration indicated the presence of steroids.

Test for alkaloids
Crude extract was mixed with 2ml of 1% HCl and heated gently. Mayer’s And Wagner’s reagents were then added to the mixture. Turbidity of the resulting precipitate was taken as evidence for the presence of alkaloids.

Spot test for iron detection
Filter paper was wet with 5% Potassium thyoocinate (Freshly prepared), dry powdered plant material were sprinkled on it and again little amount of KSCN was added after few minute red
spots were developed which shows the presence of iron in the plant material.

**Observation**

All six plants show the presence of iron among but *Centella asiatica* L. and *Cicer arietinum* L. have less quantity then rest four leaf extracts (Table:2).

### Table 1 Showing Qualitative phytochemical analysis of six plants

| Sl.no | Name of Plants             | Solvent | Alkaloids | Flavonoids | Glycosides | Phenol & Tannin | Carbohydrates | Proteins | Saponin |
|-------|----------------------------|---------|-----------|------------|------------|----------------|---------------|----------|---------|
| 1     | *Amaranthus tricolor* L.   | A       | ++        | ++         | ++         | ++             | ++            | ++       | ++      |
| 2     | *Centella asiatica* L.    | E       | ++        | ++         | ++         | ++             | ++            | ++       | ++      |
| 3     | *Chenopodium album* L.    | E       | ++        | ++         | ++         | ++             | ++            | ++       | ++      |
| 4     | *Cicer arietinum* L.      | M       | ++        | ++         | ++         | --             | ++            | ++       | ++      |
| 5     | *Colocasia esculentum* Linn. | A    | ++        | ++         | ++         | ++             | ++            | ++       | ++      |
| 6     | *Ipomia aquatic* L.       | E       | ++        | --         | ++         | ++             | ++            | ++       | ++      |

(A=Aqueous, E=Ethanolic, M=Methanolic; +++ = Strongly present, ++ = Present, + = Trace, −−− = Absent.)

### Table 2 Spot test for iron detection

| Sl. No | Name of Plants             | Spot test result of iron determination |
|--------|----------------------------|--------------------------------------|
| 1      | *Amaranthus tricolor* L.   | +++                                  |
| 2      | *Centella asiatica* L.    | ++                                   |
| 3      | *Chenopodium album* L.    | ++                                   |
| 4      | *Cicer arietinum* L.      | +++                                  |
| 5      | *Colocasia esculentum* Linn. | +++                             |
| 6      | *Ipomia aquatic* L.       | +++                                  |

(++++ Strongly present, +++ Present in less amount.)

### RESULT

Our result confirmed the presence of seven phytochemicals i.e., alkaloids, flavonoids, glycosides, carbohydrates, proteins, saponin, phenol and tannin in the extracts of six medicinal plants. Alkaloids were detected in all plants while it was strongly present in the methanolic extract of *Chenopodium album* L.(+++). Flavonoids were detected in all the plants but not observed in the ethanolic and methanolic extracts of *Chenopodium album* L. and *Ipomia aquatic* L. and methanolic extracts of *Ipomia aquatic* L. Glycosides were detected in all plants extracts. Phenol and tannins were present in all plants extracts except in aqueous extract of *Centella asiatica* L. and ethanolic extract of *Cicer arietinum* L. Carbohydrate was not detected in aqueous extracts of *Cicer arietinum* L., *Colocasia esculentum* L. and *Ipomia aquatic* L., carbohydrate was also not observed in the ethanolic and methanolic extract of *Chenopodium album* L. Protein was strongly present in ethanolic and methanolic extract of *Amaranthus tricolor* L.(++) while not observed in the methanolic extract of *Colocasia esculentum* L. Saponin is strongly present in the ethanolic extract of *Chenopodium album* L. while in methanolic extracts of all plants saponin was not detected and it was interesting to note that saponin was completely absent in all extracts of *Cicer arietinum* L. (Table: 1).

### DISCUSSION

Phytochemicals are bioactive chemical compounds present in smaller quantities in plants for their normal metabolic process, work with nutrition and dietary fiber to protect against diseases, these phytochemicals includes alkaloids, flavonoids, glycosides, terpinoids, saponin, tannin etc. and also referred as “Secondary metabolites” (Peters, 2012; Okwu, 2004).

According to American cancer society (ACS), more than 4000 phytochemicals have been cataloged and phytochemicals accumulate in different parts of the plants i.e., root, stem, leaf, seed etc. Alkaloids, flavonoids and tannins were reported for anthelmintic activity and helmintiasis is one of the causes of anemia (Yadav, et al.; 2010). Flavonoids is used for the treatment of anemia due to antioxidant activities and can cause inhibition of the oxidative modification of the human lipoproteins. (Swapana et al., 2012).

Tannin also used for the treatment of anemia and it has anti-viral, anti-bacterial and anti-parasitic activity (Liu; 2004). Carbohydrates and proteins are nutritive material.

Saponin was detected in all plant extracts except *Cicer arietinum* L. and it is used as an adjuvant in the product of vaccines. It has relationship with sex hormons like oxytocin which involves in controlling the onset of labour in women and subsequent release of milk (Okwu and Okwu; 2004).

The presence of Iron signifies that the leaves may be used against anemia and check the disorder of growth (Claude and Paule; 1979). Iron is an energizer and excess may cause fatigue (Gbolahan; 2001).

### Acknowledgement

Authors are grateful to Dr. K. Shukla, Head, P.G. Department of Botany, Kolhan University for their sincere encouragement and inspiration during their work and also to Prof. K. Boipai.

17139 | P a g e
Principal, Tata College, Chaibasa for providing necessary facilities. A special thanks to all vaidays /kabiraj /manki etc. who share their valuable knowledge with us. First author is grateful to University Grants Commission, New Delhi for the award of RGNF fellowship for financial support.

Reference

Alimoladun A.C., Ibukun O.E., Abuotu E.M., Faebomi E. (2007): Phytochemical constituents and antioxidant activity of extract from the leaves of Ocimum gratissimum. Sci. Res. Essays, 2:163-166.

American cancer society. Phytochemicals. Available at http://www.cancer.org/epirese/main/docroot/EETO/content /EETO-5-3x-phytochemicals:june 2006

Belanger J., Balakrishna M., Latha P., Katumalla S. and Johns T. (2004): “Contribution of selected wild and cultivated leafy vegetables from South India to lutein and β-carotene intake.” Asia Pacific Journal of Clinical Nutrition; in press. Johns T. andShahapit, B.R.

Buenz, I.F.F. Schenepple, D.J. and Motley, T.J. (2004): Technique, bioprospecting historical herbal; texts by hunting for new leads in old tomes. Trends in pharmacological sciences, 25, 494-498.

Claude, B. and Paule, S. (1979) : The manual of natural living.1st Ed. Biddles Ltd, pp.98-101.

Gbolahan, D.(2001): Lesson note on medical importance of trace element. Centre for Natural Health Studies, Surulere, Lagos, Nigeria.

Harborne JB. (1998): Phytochemical Methods:A Guide to Modern Techniques of Plant Analysis,3 rd Edn, Chapman and Hall Co.New York, pp.1-302.

Kattimani, K.N., P.M. Munikrishnappa, S.Abbas Hussain and P.N.Reddy (2000) :Use of plants as medicine under semi-arid tropical climate of Raichur district of North Karnataka. J. Med. Arom. Pl.Sc.22-23; 406-410.

Kuhnlein H.V. and Receveur O. (1996): “Dietary change and traditional food systems of indigenous people.” Annual review of Nutrition, 16, pp 417-442.

K.R.Kirtikar and B.D.Basu (2012): “Indian Medicinal Plants” Vol.I-VI, Lalit Mohan Basu, M.B., 49, Leader Road, Allahabad, India.

L.V. Asolkar, K.K. Kakkar and O.J.Charke, (1996): “Glossary of Indian Medicinal Plants with active principle” Part(1-A-k), National institute of Science Communication and Information resources (CSIR) Dr.K.S. Krishnan marg, Delhi..

Liu R.(2004): “Potential synergy of phytochemicals in cancer prevention. Mechanism of action.” The journal of nutrition vol.134, pp 3479-3485.

Madukwe E.U., Ugwuoke A.L. and Ezeogwu J.O. (2013): Effectiveness of dry Moringa oleifera leaf powder in treatment of anaemia. International journal of Medicine and Medical Sciences; 5(5), pp. 226-228.

Mahato, S.B.: Sen, S.(1997): Advances in triterpenoid research, 1990-1994. Phytochemistry, 44:1185-1236.

Oladiji AT. (2003): Tissue levels of Iron, Copper, Zinc and Magnesium in iron deficient rats. Biochemistri. 14: 75-81.

Okeno JA, Chebter DK (2003): Mattages of Indigenous Vegetables in Kenya. Acta Horticult.621:93-100.

Okwu, D.E., (1999): Flavouring properties of species on cassava futsu. Afr. J. Roots Tubes crops 3(2):19-21.

Okwu D.E. (2001): Evaluation of the chemical composition of indigenous spices and flavouring Agents. Global J.pure Appl.sci.7 (3):455-459.

OkwuD.E., Okwu, M.E.(2004): Chemical composition of spondiasmombin Linn. plant parts. J.sust. Agric. Environ 6:140-147.

Peteros, N.P. (2010): VYMM. Antioxidant and cytotoxic activities and phytochemical screening of four phillppine medicinal plants. J.Med.Plant.Res.8(5):407-414.

R.N.Chopra, S.L.Nayar and I.C. Chopra, (1996): “Glossary of Indian Medicinal Plants,” National institute of Science Communication and Information resources (CSIR) Delhi, India.

Ruckmani, K., Kavimani, S., Anandan, R.and Jaykar, B.(1998): Effect of moringa leaf Lam. On paracetamol induced hepatotoxicity. Ind. J. Pharmaceutical Sci.:60:33-35.

Sofowora A, (1993): Medicinal plant and Traditional Medicine in Africa. Published by wiley and sons Limited Chichester, pp 256.

Swapana, N., Jotinkumar, T., Devi, C.B., Singh, M.S., Singh, S.B., et.al. (2012): Total phenolic, total flavonoid contents and antioxidant activity of a few indigenous fruit grown in Manipur. The bio.scan 7:73-76.

Tress G.E., Evans W.C. A (1956): Text book of Pharmacognosy.14 th Edn, Bailliere Tindall Ltd. London.

Ughade,S.N., Zodpey S.P. and Khanolkar V.A.,(1998): “Risk factors of cataract: a case control study.” Indian Journal of Ophthalmology, 46(4): pp 221-227

Yadav,P., Kumar, A., Mahour K., and Vihan,V.S.(2010): Phytochemical Analysis of some Indigienous Plants Potent Against Endoparasite. Journal of Advanced Laboratory Research in Biology, 1(1), pp.56-59.

World Health Organization (WHO). (1968): Nutritional Anaemias. WHO technical reoort series. 402 Geneva. World Health Organization.

How to cite this article:

Dolly Tudu and Vishnu Shankar Sinha.2017, Qualitative Phytochemical Analysis of some Leafy Vegetables used to Cure Anemia in west Singhbhum, Jharkhand, India. Int J Recent Sci Res. 8(5), pp. 17137-17140.

DOI: http://dx.doi.org/10.24327/ijrsr.2017.0805.0290

 ********