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Research Article

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

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ARTICLE INFO	ABSTRACT			
<i>Article History:</i> Received 18 th February, 2017 Received in revised form 10 th March, 2017 Accepted 06 th April, 2017 Published online 28 th May, 2017	Present study reports the preliminary qualitative phytochemical analysis of six medicinal plants <i>viz.</i> <i>Amaranthus tricolor</i> L. (Amaranthaceae), <i>Centella asiatica</i> L.(Apiaceae), <i>Chenopodium album</i> L.(Amaranthaceae), <i>Cicer arietinum</i> L.(Fabaceae), <i>Colocasia esculentum</i> L.(Araceae) and <i>Ipomia aquatic</i> 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			
Key Words:	spot was tested by 5% potassium thyocinate solution (KSCN).			

Our result confirmed the presence of major classes of photochemical 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.

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INTRODUCTION

Ethnomedicine, Phytochemical,

Qualitative, Secondary metabolites.

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 (Alinmoladun *et. al.*, 2007). In pregnant and nursing mothers plants leaves are used for medicinal and nutrirional 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 practinoer and traditional medicines for their primary health care. Traditional knowledge provides clues to the discovery of valuable druges (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, hepatopeotective, 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 anemia by tribal and rural people of West Singhbhum, Jharkhand, India.

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 grounded 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 the 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 diluted 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 and2ml 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., glyconeportion 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., glycone 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 thyocinate (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.

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

Observation

					Phy	tochemicals			
Sl.no	Name of Plants	Solvent	Alkaloids	Flavonoids	Glycosides	Phenol & Tannin	Carbohydr ates	Proteins	Saponi n
	Amaranthus tricolor	А	++	++	++	++	++	++	++
1	1 Amaraninus incolor L.	Е	++	++	++	++	++	+++	+
		М	++	+	++	+	++	+++	
		А	++	++	++		++	++	
2	Centella asiatica L.	Е	++	++	++	++	++	++	++
		М	++	++	++	++	++	++	++
	Chananadium	А	++	++	++	++	++	++	+ ++
3	Chenopodium album L.	Е	++		++	++		++	+++
	uibum L.	М	+++		++	++		+	
		А	++	++	++	++		++	
4	Cicer arietinum L	Е	++	++	++		++	+	
		М	++	+	++	++	++	+	
	Colocasia	А	++	++	++	++		++	++ ++
5	esculantum Linn.	Е	++	++	++	++	++	+	
	escuuntum Enni.	М	++		++	++	++		
		А	++	+	++	++		++	+
6	Ipomia aquatic L.	Е	++		++	++	++	++	++
		М	++		++	++	++	++	++

Table 1 Showing Qualitative phytochemical analysis of six plants

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

 Table 2 Spot test for iron detection

SI. 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 esculantum 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 pdetected 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 esculantum 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 esculantum 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" (Peteros, 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 helminthiasis 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 antiviral, 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).

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