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Qualitative Analysis Of Phytochemicals, Vitamins And Inorganic Elements Of *Curcuma Amada* Rhizome And *Tagetes Erecta* Flower Extracts

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ABSTRACT

Research regarding medicinal plant is a highlighted issue today. In the present study was to investigate the qualitative analysis of phytochemicals, vitamins and inorganic elements of *Curcuma amada* rhizome and *Tagetes erecta* flower extract. Phytochemicals such as saponin, flavonoids, terpenoids and polyphenol present in *Curcuma amada* rhizome and *Tagetes erecta* flower. The tannin, Phlobatannins, steroids, triterpenoid and protein were absent in *Curcuma amada* rhizome and *Tagetes erecta* flower extract. The significant amount of Phenol, Flavonoids and Saponin were present. Histochemical study further confirmed in the presence of phytochemicals in the plant. The elements such as Calcium, Magnesium, Sodium, Potassium, Sulphate, Phosphate, Chloride, and Nitratewere found to be in *Tagetes erecta flower* and *Curcuma amada* rhizome extracts. Vitamin A, D and E. Vitamin C was present in Tagetes *erecta* flower while Vitamin C was absent in *Curcuma amada* rhizome extract.

KEYWORDS: Phytochemicals, Vitamins, Inorganic elements, *Curcuma amada* rhizome and *Tagetes erecta* flower

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INTRODUCTION

Research regarding medicinal plant is a highlighted issue today. According to World Health Organization (WHO) more than 80% of the world's population, mostly in poor and less developed countries depend on traditional plant-based medicines for their primary healthcare needs. Plant and plant products are being used as a source of medicine since long. Medicinal plants are the nature's gift to human being to make disease free healthy life. It plays a vital role to preserve our health. India is one of the most medico-culturally diverse countries in the world where the medicinal plant sector is part of a time-honored tradition that is respected even today. Owing to the global trend towards improved 'quality of life', there is considerable evidence of an increase in demand for medicinal plant. ¹

Many of today's synthetic drugs originated from the plant kingdom but, historically, medicinal herbalism went into decline when pharmacology established itself as a leading and effective branch of medical therapeutics. In much of the English-speaking world, herbalism virtually vanished from the therapeutic map of medicine during the last part of the 19th and early part of the 20th century. However, in many third world countries various forms of ethnic herbalism prevail to the present day (e.g., Ayurvedic medicine in India, Kampo medicine in Japan, and Chinese herbalism in China). In some developed countries, (e.g., Germany and France), medical herbalism continues to co-exist with modern pharmacology, albeit on an increasingly lower key. Medicinal plants are the nature's gift to human being to make disease free healthy life. It plays a vital role to preserve our health. India is one of the most medico-culturally diverse countries in the world where the medicinal plant sector is part of a time-honored tradition that is respected even today. Traditional medicines derive their scientific heritage from rich experiences of ancient civilization. Hence, it is not surprising that traditional medicines claim comes for several "difficult to cure". In the present study was to investigate the qualitative analysis of phytochemicals, vitamins and inorganic elements of *Curcuma amada* rhizome and *Tagetes erecta* flower extracts.

MATERIALS AND METHODS

Collection of Plant materials

During the month of February, *Curcuma amada* rhizome and *Tagetes erecta* flowers were collected from various gardens in Keelavandanviduthy Village, Pudukkottai district, Tamil Nadu, India.

Authentication of plants

The plant was identified and carefully examined with the help of region floras. Specimens were further confirmed with reference to herbarium sheets available in the Rapinat Herbarium, St, Joseh's College, Tiruchirappalli, Tamil Nadu, India. **Preparation of extracts**

The powdered rhizome and flowers material (20 g) was soaked in 50 ml of 70% Methanol for 12 hours and then filtered through a Whatmann filter paper along with 2 g sodium sulphate to remove the sediments and traces of water in the filtrate. Before filtering, the filter paper along with sodium sulphate was wetted with absolute alcohol. The filtrate is then concentrated by bubbling nitrogen gas into the solution and was concentrated to 1 ml. The extract contains both polar and non-polar phytocomponents.

Histochemical tests

The powder of *Curcuma amada*rhizome and *Tagetes erecta flowers* were treated with specific chemicals and reagents. The *Curcuma amada* rhizome and *Tagetes erecta flowers* treated with phloroglucinol and diluted HCl gave red colour indicates lignin, treated with diluted ammonia and H₂SO₄ gave yellow colour indicates flavanoids, treated with iodine solution gave blue colour indicates starch and treated with ferric chloride gave blue green colour indicates tannins and phenols. The treated plant powder further analysed in light microscope.

Preliminary phytochemical screening

Preliminary phytochemical evaluation was carried out by using standard procedure. 4-7 Qualitative analysis of vitamins. 8-9

Qualitative analysis of inorganic elements

Ash of drug material (500mg) was prepared and treated with HNO_3 and HCl (3:1 v/v) for 1 hour. After the filtration, the filtrate was used to perform the following tests. ¹⁰

Quantitative determination of the chemical constituency

Determination of total phenols by spectrophotometric method: Tannin determination by Van-Burden and Robinson ¹¹ method: Flavonoid determine by the method of Bohm and Kocipai-Abyazan, ¹²

RESULTS

Now-a-days there is a renewed interest in drugs of natural origin simply because they are considered as green medicine and green medicine is always supposed to be safe. Another factor which emphasizes this attention is the incidences of harmful nature of synthetic drugs which are regarded as harmful to human beings and environment. The advantage of natural drugs is their easy

availability, economic and less or no side effects but the disadvantage is that they are the victims of adulteration. ¹³

The first step towards ensuring quality of starting material is authentication. Thus, in recent years there has been a rapid increase in the standardization of selected medicinal plants of potential therapeutic significance. Despite the modern techniques, identification of plant drugs by pharmacognostic studies is more reliable. The standardization of crude drugs is important before any work carried out. Therapeutic efficacy of medicinal plants depends upon the quality and quantity of chemical constituents. The misuse of herbal medicine or natural products starts with wrong identification. The physicochemical test is the first step towards establishing the identity and the degree of purity of such materials and should be carried out before any tests are undertaken. ¹⁴

In the present study to investigatae the *Curcuma amada* rhizome and *Tagetes erecta* flower in physico chemical analysis, phytochemical screening, *In vitro* antioxidant activity, anti-inflammatory and larvacidal activity.

Phytochemical screening of Curcuma amada rhizome and Tagetes erecta flower extract

The qualitative analysis of ethanolic extract of *Curcuma amada* rhizome and *Tagetes erecta* flower investigated and reprented in Table 1. Phytochemicals such as saponin, flavonoids, terpenoids and polyphenol present in *Curcuma amada* rhizome and *Tagetes erecta* flower. The tannin, steroids, Phlobatannins, triterpenoid and protein were absent in *Curcuma amada* rhizome and *Tagetes erecta* flower extract.

The quantitative analysis of *Curcuma amada* rhizome contains Phenol (9.3 mg/gmpowder), Flavonoids (6.42 mg/gm powder) and Saponin (4.84mg/gm powder) (Table 4.2). The quantitative determinations of *Tagetes erecta* flower contain Phenol (8.4 mg/gm powder), Flavonoids (5.86 mg/gm powder) and Saponin (4.24mg/gm powder) (Table 2).

Table.1: Preliminary Qualitative phytochemical screening of *Curcuma amada* rhizome and *Tagetes erecta* flower extract

S. No.	Phytochemical	Results		
		Curcuma amadarhizome	Tagetes erecta flowers	
1.	Tannin	_	_	
2.	Phlobatannins	_	_	
3.	Saponin	++	++	
4.	Flavonoids	++	++	
5.	Steroids	++	++	
6.	Terpenoids	+	+	
7.	Triterpenoids	_	_	
8.	Alkaloids	++	_	
9.	Carbohydrate	_	+	
10.	Protein	_	_	
11.	Anthroquinone	_	+	
12.	Polyphenol	++	++	
13.	Glycoside	_	++	

(+) Indicates Present

(--) Indicates Absent (++) Moderately present

Table.2: Quantitative analysis of Tagetes erecta flower and Curcuma amada rhizome extracts

S. No	Quantitative Analysis	Curcuma amada rhizome (mg/5gm)	Tagetes erecta flower (mg/5gm)
1	Flavonoids	184.00±12.88	168.00±11.76
2	Phenols	344.00±24.08	314.66±21.98
3	Tannin	104.00±7.28	88.00±6.16

Values are expressed as Mean \pm SD for triplicates

Histochemical analysis of of Curcuma amada rhizome and Tagetes erecta flower powder

The powder of *Curcuma amada rhizome and Tagetes erecta flower powder*were treated with specific chemicals and reagents. The *Curcuma amada* rhizomeand *Tagetes erecta* flower *powder*es powder treated with phloroglucinol and diluted HCl gave red colour indicates lignin, treated with diluted ammonia and H₂SO₄ gave yellow colour indicates flavanoids and treated with Toludine Blue gave blue colour indicates Polyphenol. The treated plant powder further analysed in light microscope (Table 3).

Table.3: Histochemical analysis of of Curcuma amada rhizome and Tagetes erecta flower powder

S.No	Test reagents	Observations	Present	Curcuma amada rhizome	Tagetes erecta flowers
1.	Phloroglucinol + concentrate Hcl	Red/Pink	Lignin	+	+
2.	Dilute Ammonia + H ₂ SO ₄	Yellow	Flavonoids	+	+
3.	FeCl ₃ Solution (few drops)	Dark Blue to Black	Tannin	+	+
4.	Iodine (few drops)	Blue	Starch grain	+	+
5.	Toludine Blue	Blue Green / Red	Polyphenol	+	+

(+) Indicates Present

(--) Indicates Absent

Qualitative Analysis of Inorganic Elements in *Tagetes erecta* flower and *Curcuma amada* rhizome extracts

The following elements were found to be in *Tagetes erecta flower* and *Curcuma amada* rhizome extracts. They are Calcium, Magnesium, Sodium, Potassium, Sulphate, Phosphate, Chloride, and Nitrate (Table 4).

Table.4: Qualitative inorganic elemental analysis of Curcuma amada rhizome and Tagetes erecta flower

S. No.	Analysis	Curcuma amada rhizome	Tagetes erecta flowers
1	Calcium	+++	+++
2	Magnesium	+++	++
3	Sodium	+	+
4	Potassium	+++	+
5	Iron	+++	+++
6	Sulphate	+++	+++
7	Phosphate	+	+
8	Chloride	+++	+++
9	Nitrate	+	+++

(+) Indicates Present

(--) Indicates Absent (++) Moderately present

Qualitative Analysis of Inorganic Elements in *Tagetes erecta* flower and *Curcuma amada* rhizome extracts

The following vitamins were found to be in *Tagetes erecta flower* and *Curcuma amada rhizome* extracts. They are Vitamin A, D and E. Vitamin C was present only in Tagetes *erecta* flower while *Curcuma amada* rhizomeextract were absent (Table 5).

Table.5: Qualitative vitamin analysis of Curcuma amada rhizome and Tagetes erecta flower

S. No	Analysis	Curcuma amada Rhizome	Tagetes erecta flowers
1	Vitamin-A	+	+
2	Vitamin-C		+
3	Vitamin-D	+	++
4	Vitamin-E	+	++

(+) Indicates Present

(--) Indicates Absent (++) Moderately present

DISCUSSION

Plants have basic nutritional importance by their content of protein, carbohydrate, fats and oils minerals, vitamins and water responsible for growth and development in man and animals. Phytochemical simply means plant chemicals. "Phyto" is the Greek word for plant. Phytochemicals

are classified as primary or secondary constituents, depending on their role in plant metabolism. Primary metabolism is important for growth and development of plants include the common sugars, aminoacids, proteins, purines and pyrimidines of nucleic acids, chlrophyll's etc. Secondary metabolism in a plant plays a major role in the survival of the plant in its environment. Attractions of pollinators, natural defense system against predators and diseases, etc., are examples of the roles of secondary metabolites. ⁴

The secondary metabolites formed also are an important trait for our food plants (taste, colour, scent, etc.) and ornamental plants. Moreover, numerous plant secondary metabolites such as flavonoids, alkaloids, tannins, saponins, steroids, anthocyanins, terpenoids, rotenoids etc. have found commercial application as drug, dye, flavour, fragrance, insecticide, etc. Such fine chemicals are extracted and purified from plant materials.¹⁵

Qualitative and Quantitative Analysis

Plants have basic nutritional importance by their content of protein, carbohydrate, fats and oils minerals, vitamins and water responsible for growth and development in man and animals. The qualitative analysis of ethanolic extract of *Curcuma amada* rhizome and *Tagetes erecta* flower investigated and reprented in Table 1. Phytochemicals such as saponin, flavonoids, terpenoids and polyphenol present in *Curcuma amada* rhizome and *Tagetes erecta* flower. The tannin, Phlobatannins, steroids, triterpenoid and protein were absent in *Curcuma amada* rhizome and *Tagetes erecta* flower extract.

The quantitative analysis of *Curcuma amada* rhizome contains Phenol (9.3 mg/gm powder), Flavonoids (6.42 mg/gm powder) and Saponin (4.84mg/gm powder). The quantitative analysis of *Tagetes erecta* flowers contain Phenol (8.4 mg/gm powder), Flavonoids (5.86 mg/gm powder) and Saponin (4.24mg/gm powder). The results of the present study agreement with Enas Mehjen Numan *et al.* ¹⁶ and Kulkarni *et al.* ¹⁷ studies. The results of the present study concluded that alcoholic extract of *Curcuma amada* rhizome and *Tagetes erecta* flowers showed that saponin, flavonoids, terpenoids and polyphenol in both extract. Over all, *Curcuma amada* rhizome and *Tagetes erecta* flowers contains rich source of phytochemicals which are important in diseases prevention.

Histochemical analysis of Curcuma amada rhizome and Tagetes erecta flower powder

Histochemistry is the branch of histology dealing with the identification of chemical components of cells and tissues, it is a powerful tool for localization of trace quantities of substances present in biological tissues. Histochemical techniques have been employed to characterize structure

and development, and to study time course of deposition and distribution of major storage compounds such as proteins, lipids, starch, phytin and minerals like calcium, potassium and iron.¹⁸ The importance of histochemistry in solving critical biosystematic problems is as popular as the use of other markers. According to botanical literatures, the use of histochemical characters in taxonomic conclusions is now a common practice.

Results of the present study revealed that *Curcuma amada* rhizome and *Tagetes erecta* flowers powder treated with phloroglucinol and diluted HCl gave red colour indicates lignin, treated with diluted ammonia and H₂SO₄ gave yellow colour indicates flavanoids and treated with Mayers reagent gave reddish brown colour indicates alkaloids. The treated plant powder further analysed in light microscope. The following review supported our studies.

John Peter Paul ¹⁹ attempt was taken for histochemical and fluorescence analysis of *Turbinaria ornata* (Turner). Histochemical analyses of the plant were carried out using light microscopy and fluorescence study was analyzed by UV lamp. Results of histochemical tests showed positive reaction to phenol compounds, polyphenol and tannin in the thallus. Fine powder and different solvent extracts of *Turbinaria ornata* obtained using petroleum ether, benzene, chloroform, acetone, ethanol and aqueous were examined under visible and UV light. The powdered materials were also treated with various reagents such as 50% nitric acid, 50% sulphuric acid, 1N HCl, 1N NaOH and changes in colour were recorded. He concluded that the histochemical and the fluorescence analysis could be used for rapid identification of potential medicinal plants and bioactive compounds which is present in the particular plant.

Qualitative Analysis of Inorganic Elements in *Curcuma amada* rhizome and *Tagetes erecta* flower

All human beings require a number of complex organic/inorganic compounds in diet to meet the need for their activities. The important constituents of diet are carbohydrates, fats, proteins, vitamins, minerals and water. Every constituent plays an important role and deficiency of any one constituent may lead to abnormal developments in the body. Plants are the rich source of all the elements essential for human beings. There is a relationship between the element content of the plant and its nutritional status. Some elements are essential for growth, for structure formation, reproduction or as components of biologically active molecules while others have some other beneficial effects. ²⁰

Qualitative or quantitative determination of mineral elements present in plants is important because the concentration and type of minerals present must often be stipulated on the label of a food. The quality of many foods depends on the concentration and type of minerals what they

contains, also play a very significant role against a variety of degenerative diseases and processes, they may also prevent and reduce injury from environmental pollutants and enhance the ability to work and learn, some minerals are essential to a healthy diet (e.g. Calcium, Phosphorus, Potassium and Sodium) where as some can be toxic (e.g. Lead, Mercury, Cadmium and Aluminium). It is clear that mineral nutrition is important to maintain good health and because of that determination of As, Ca, Fe, Mg, Na, K, Zn, Ni, Co etc. have been added to Ayurvedic Pharmacopoeia of India.²¹ From ancient times, Swarnabhasma (gold ash) has been used in several clinical manifestations including loss of memory, defective eyesight, infertility, overall body weakness and incidence of early aging. Hence, their presence is vital for the health and to cure diseases. Mineral content indicates the nutritive value and potentially act as a cofactor for the biological activity exhibited by the plant extracts studied.

The following elements were found to be in *Tagetes erecta flowern* and *Curcuma amada rhizome extracts*. They are Calcium, Magnesium, Sodium, Potassium, Sulphate, Phosphate, Chloride, and Nitrate. Carbonate was absent in all plants. Mineral content indicates the nutritive value and potentially act as a cofactor for the biological activity exhibited by the plant extracts studied.

Qualitative Analysis of Vitamins in Curcuma amada rhizome and Tagetes erecta flower

Vitamins are organic substances that are essential in tiny amounts for growth and activity of the body. They are obtained naturally from plant and animal foods. Organic in this definition refers to the chemistry and molecules of vitamins. The word organic means that the molecules of the substance contain the element carbon. The term also means that vitamins can be destroyed and become unable to perform their functions in our bodies. Too much heat, certain kinds of light and even oxygen can destroy some vitamins. The amounts of vitamins ingested from food are measured in micrograms or milligrams.²²

Vitamins work with other substances in the body like enzymes and minerals. Together they perform such functions as strengthening bones, healing wounds, keeping the skin healthy, building cells, and helping to resist infections. Vitamins are separated into two groups, fat soluble and water soluble. The fat soluble vitamins are A, D, E, and K, and can dissolve in dietary fats and are stored in the liver and body fat. The body stores them for a longer amount of time, so they are not needed every day. Too much of these vitamins can become toxic and cause health problems. The water soluble vitamins are made up of 8 B vitamins and vitamin C. Water soluble vitamins dissolve in water, and are not stored in the body. Rather they travel through the bloodstream and need to be replenished every day. These vitamins are easily destroyed during food preparation and storage.

Vitamin A, also known as retinol, is needed for skin and body tissue repairs. Children need vitamin A to build bones and teeth. Vitamin A is part of the body's defense system against infections. Vitamin A deficiency is a problem in countries where people eat very few dairy products, fruits, or vegetables. One of the first signs of a vitamin A deficiency is difficulty seeing at night because the retina of the eye needs the vitamin to function well. Many vegetables also supply vitamin A, such as carrots, pumpkins, and squash; as well as the yellow fruits such as cantaloupes and peaches. Dark green vegetables, tomatoes, and sweet potatoes are also a good source. Most of these fruits and vegetables do not actually contain vitamin A, but rather contain beta carotene which the body converts to vitamin A. Carotene is the pigment that makes egg yolks yellow and carrots bright orange. Most adults carry enough vitamin A in their livers to supply them for months. Large doses of vitamin A can cause liver damage, and this is why most multi-vitamin supplements have some of the vitamin A come from beta carotene rather than retinol. Many studies have made claims that beta carotene in fruits and vegetables helps reduce the risk of some cancers.²³

Vitamin D is important in bone formation. Most vitamin D is made when sunshine hits the skin. Too much sun can contribute to skin cancer, and using a sunscreen of SPF 15 or more will block vitamin D formation. Milk and margarine are both fortified with vitamin D. Those over the age of 65 only make about half as much vitamin D as children from the same amount of light exposure, so it is recommended to take a supplement for these people to get enough vitamin D. A vitamin D deficiency can cause an older disease called rickets, and it is cured by cod-liver-oil, which has a high concentration of vitamin D. Vitamin D is stored in the liver and as little as 5 times the Daily Value can produce unhealthy weight loss, vomiting, and calcium deposits in the lungs and kidneys.²⁴

Vitamin E remains the most mysterious of vitamins. The body needs it but its lack does not lead to any known disease. Vitamin E is the most exploited vitamin in that it is sold as a cure-all and even as an anti-aging potion. Vitamin E, vitamin C, and beta carotene are antioxidants. Some studies suggest that the trio might help to strengthen the body's immune system and play a role in cancer prevention. ²⁵

Vitamin C, or ascorbic acid, is one vitamin humans cannot synthesis and they have to get it from food. Vitamin C helps hold the cells together, heal wounds, and build bones and teeth. The best sources for vitamin C are citrus fruits, strawberries, melons, and leafy green vegetables. Vitamin C also helps to absorb and use Iron. It is important to protect the vitamins in fruits and vegetables from being destroyed; simple ways of doing this include refrigeration, washing them before cutting them, storing them in airtight containers, and avoiding high temperatures and long cooking times.²⁵

CONCLUSSION

Phytochemicals such as saponin, flavonoids, terpenoids and polyphenol present in *Curcuma amada* rhizome and *Tagetes erecta* flower. The tannin, Phlobatannins, steroids, triterpenoid and protein were absent in *Curcuma amada* rhizome and *Tagetes erecta* flower extract. The quantitative analysis of *Curcuma amada* rhizome contains Phenol (9.3 mg/gm powder), Flavonoids (6.42 mg/gm powder) and Saponin (4.84mg/gm powder) while *Tagetes erecta* flower contain Phenol (8.4 mg/gm powder), Flavonoids (5.86 mg/gm powder) and Saponin (4.24mg/gm powder). The *Curcuma amada* rhizome and *Tagetes erecta* flower *powder*es powder treated with phloroglucinol and diluted HCl gave red colour indicates lignin, treated with diluted ammonia and H₂SO₄ gave yellow colour indicates flavanoids and treated with Toludine Blue gave blue colour indicates Polyphenol. This study further confirmed in the presence of phytochemicals in the plant. The elements such as Calcium, Magnesium, Sodium, Potassium, Sulphate, Phosphate, Chloride, and Nitratewere found to be in *Tagetes erecta flower* and *Curcuma amada* rhizome extracts. Vitamin A, D and E. Vitamin C was present in Tagetes *erecta* flower while Vitamin C was absent in *Curcuma amada* rhizome extract.

REFERENCES

- 1. WHO. Quality control methods for medicinal plant materials. Geneva: Word Health Organization. 1998;25-36.
- 2. Gao ZK, Huang X, Yang Hxu. Free radical scavenging and antioxidant activities of flavonoids extracted from the radix of Scutellariabaicalensis Georgi. Biochimica Biophysicsa Acta. 1999;472, 643-650.
- 3. Satyavati GV. Some traditional medical systems and practices of global importance. Indian Journal of Medical Research. 1982, 76,1-5.
- 4. Sofowara A. Medicinal plants and Traditional medicine in Africa. Spectrum Books Ltd, Ibadan, Nigeria. 1993; 289.
- 5. Trease GE, Evans WC. Phenols and Phenolic glycosides. In: Textbook of Pharmacognosy. (12th ed.). Balliese, Tindall and Co Publishers, London 1989; pp. 343-383
- 6. Edeoga HO, Omusun LC, Uche K. Chemical composition of Hyptis suavelens and Ocimum gratissimum hybrids from Nigeria, African journal of biotechnology, 2006; 5(10), 892-895.
- 7. Harborne JB. Phytochemical methods. A Guide to modern techniques of plant analysis 2nd edition. Chapman and Hall, London, 1984; 4-120.

- 8. Patel KK. Master dissertation. Shorea robustra for burn wound healing and antioxidant activity. Department of Pharmacology, KLESS College of Pharmacy, Karnataka, India, 2005; 33.
- 9. Pearson D. The Chemical Analysis of Food, 17th ed. Churchill Livingstone, London.. 1976pp 3-4.
- 10. Khandelwal KR. Practical Pharmacognosy (16th ed.,) Nirali Prakashan, Pune, 2006; 98-106
- 11. Van-Burden TP and Robinson WC. Formation of complexes between protein and Tannin acid. *J. Agric. Food Chem*, 1981; 1: 77.
- 12. Bohm BA, Kocipai-Abyazan R. Flavonoids and condensed tannins from leaves of Hawaiian vaccinium vaticulatum and V calycinium. Pacific Sci, 1994; 48: 458-463.
- 13. Dineshkumar C. Pharmacognosy can helpminimize accidental misuse of herbal medicine. Curr Sci, 2007; 3:1356-1358.
- 14. Tatiya A, Surana S, Bhavsar S, Patil, Patil Y. Pharmacognostic and preliminary phytochemical investigation of Eulophia herbacea Lindl. Tubers (Orchidaceae). Asian Pac J Trop Disease, 2012; 2(1):50-55.
- 15. Das K, Tiwari RKS, Shrivastava DK. Techniques for evaluation of medicinal plant products as antimicrobial agent: Current methods and future trends. Journal of Medicinal Plants Research, 2010; 4(2), 104-111.
- 16. Kulkarni R., Prakashchandra F. Khatwani and Vanita Gurale. Study of Antibacterial and Antifungal Activity Of Photoactivated Thiophene Derivatives from the Roots of Tagetes Erecta Savita. Int J Pharm Bio Sci.; 2016; 7(2): (P) 315 326.
- 17. Enas Mehjen Numan, Jamal Salman Jyad, Ahmed H. Ibrahim Alazawi, Essam F. Al-Jumaily, Atlal Nayeff Jwad, Hashim Mohammed Zehrawo, Abeer Fayik Kamel, Osama Abdulmunem Khayri. Comparison of Different Extraction Methods of (Zingiber officinale) on Chemical Composition, Antioxidant Activity. Ijppr.Human, 2016; 5 (3): 215-223.
- 18. Krishnan S, Ebenezer GAI, Dayanandan P. Histochemical localization of storage components in Caryopsis of rice (Oryza sativa L.) Curr Sci, 2001; 80: 567-571.
- 19. John Peter Paul J. Histochemistry And Fluorescence Analysis Of Turbinaria Ornata (Turner) J.Ag.—An Imp)ortant Brown Seaweed (Phaeophyceae). Indian Journal of Plant Sciences, 2014; 3 (1): 40-44.
- 20. NewWall CA, Anderson LA, Phillipsan JD. Herbal medicines- A guide for healthcare professionals. The Pharmaceutical Press, London. 1996.
- 21. The Ayurvedic Pharmacopoeia of India. Government of India, Ministry of health and family welfare, Department of Indian system of medicine and homeopathy 1999; 1: 1st

- 22. Okwu DE. Investigation into the medicinal and nutritional potential of garcinia Kala (Britter kala) Heckel and Demettia tripetala G.Beaker Ph.D. Dissertation of Michael Okpara University of Agriculture Umudlike, Nigeria. 20035-10.
- 23. Duffy Roberta. American Dietetic Association's Complete Food and Nutrition Guide, Third Edition. Wiley Publishing, Inc. 2006.
- 24. Clark Nancy and Nancy Clark's. Sports Nutrition Guide Book, Fourth Edition. Champaign: Human Kinetics 2008.
- 25. Okwu DE. Phytochemical and vitamin content of indigenous spices of south eastern Nigeria. J. Sustain Adric. Environ. 2004; 6(1): 30-37.