PHYTOCHEMICAL INVESTIGATION AND *IN VITRO* ANTICANCER ANALYSIS OF *CYCLEA PELTATA* (LAM) HOOK. F. & THOMSON OF MENISPERMACEAE

Pradeesh S.*, Sukritha V. S. and Aswathi Krishna K. U.

Received: 15/2/2023 **Revised: 15/5/2023** **Accepted: 19/5/2023** **Published: 30/12/2023**

Abstract
Plants are considered as the rich source of minerals and nutrients. The natural products obtained from the plant provide many health benefits to all living organisms. Many medicineswere prepared to cure deadly diseases like cancer. They less toxic, free from side effects comparatively safe, eco-friendly and easily available. *Cyclea peltata* (Lam) Hook. F.& Thomson, commonly known as "Indian Moon -seed" is a twining shrub belongs to family Menispermaceae. The plant has large medicinal importance and is widely distributed in the world. In the present study phytochemical, nutritional, antioxidant and anticancer activities ofplant were carried out. In the preliminary phytochemical analysis reducing sugar, alkaloids, tannins, steroids, saponins, flavonoids, anthraquinones and terpenoids were qualitatively analysed. From the result it was found that major phytochemicals are present in the crude methanolic leaf extract of *Cyclea peltata.* The different nutritional factors like reducing sugar,total carbohydrates, total protein, pigments and starch were analysed by standard estimation method and it was found to be higher. The antioxidants like proline, lycopene, lipid peroxide, total polyphenol, amylase, polyphenol oxidase (PPO) and super oxide dismutase (SOD) were also estimated quantitatively and found to be high in *Cyclea peltata.* Anticancer activity of crude methanolic leaf extract were analysed using DLA (Dalton's Lymphoma Ascites) and EAC (Ehrlich Ascites Carcinoma) cell lines and found very promising result. The result is applicable for further pharmacological analysis of the plant and isolation of new drug from theplant. Nutritional and phytochemical information on the plant will be useful for the nutritionaleducation of the public as a means to improve the nutritional status of population.

Keywords: *Cyclea peltata*, superoxide dismutase, polyphenol oxidase, DLA and EAC.

Introduction

India is a varietal emporium of medicinal plants and is one of the richest countries in the world in regard to genetic resources of medicinal plants. It exhibits a wide range in topography and climate, which has a bearing on its vegetation and floristic composition. Moreover, the agro-climatic conditions are conducive for introducing and domesticating new exotic plant varieties (Martins *et al*., 2001). Therefore, researchers are increasingly turning their attention to folk medicine, looking for new leads to develop better drugs against microbial infections (Benkeblia, 2004). The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the screening of several medicinal plants for their potential antimicrobial activity (Iwu *et al*., 1999). In recent years, secondary plant metabolites (phytochemicals), previously with unknown pharmacological activities, have been extensively investigated as a source of medicinal agents(Krishnaraju *et al*., 2005). Thus, it is anticipated that phytochemicals with adequate antibacterial efficacy will be used for the treatment of bacterial infections (Balandrin *et al*., 1985). Since time immemorial, man has used various parts of plants in the treatment and prevention of various ailments (Tanaka *et al.,* 2002).

Cyclea peltata belongs to family Menispermaceae is a slender twining herb. They are used for medicinal purpose, both, internally as well as externally. External application of the

*Post Graduate Department and Research Centre of Botany, Mahatma Gandhi College, Kesavadasapuram, Thiruvananthapuram, Kerala, India -695 011.
email : pradeeshnair10@gmail.com (Corresponding author)

paste of its roots and leaves is extremely beneficial, in infected wounds, sinuses and skin diseases like erysipelas and pruritus. The external application of this paste is said to be useful in serpent bite also. The root juice is salutary in headache, as nasal drops. The roots have anti-inflammatory activity and hence alleviated the edema. *Cyclea peltata* is a valuable wound healer and antidermatosis herb. Even though this plant is used as food and medicine. However,no much scientific validation has been made for this species for its medicinal uses. To address this lacuna, the present study was carried out for qualitative phytochemical analysis, nutritional evaluation and evaluation of anticancer property of the crude methanolic leaf extract in DLA and EAC cell line . In future *Cyclea peltata* is important natural source of developing new drugs. So have selected the plant for the present study.

Materials and methods

Collection and Preparation of Sample for Phytochemical Analysis

The plant *Cyclea peltata* was collected as fresh from Pachamala, Thiruvananthapuram district of Kerala. For sample preparation, fresh leaves were separated, shade dried, ground well using mechanical blender to fine powder and transferred into airtight container for further analysis.

Extraction from Plant Parts

The fine powder was used for extraction by methanol solvent. Fifty grams of sample powder kept into the soxhlet apparatus for distillation. Methanol was taken in the round bottom flask. The apparatus was kept over heating mantle and heated for 8 hours at 70°C. After completing the process, extract was collected in a beaker and was kept in oven at 37°C-40°C. The crude concentrated extract was again weighted and used for further biochemical analysis and anticancer analysis by using DLA and EAC cell lines.

Phytochemical analysis

The phytochemical analysis of plant extract was done as described by Harborne (Harborne, 1977). The phytochemical analysis like reducing sugar, flavonoids, alkaloids, tannins, terpenoids, steroids, saponins and anthraquinones were tested.

Biochemical analysis

The fresh leaves of *Cyclea peltata* were used for the nutritional and antioxidant analysis and experiment was repeated thrice to confirm the result. The analysis was preformed following standard methods for estimation of reducing sugar (Miller, 1972), total carbohydrate (Hedge and Hofreiter, 1962), total protein (Lowry *et al*., 1951), chlorophyll (Witham *et al*., 1971), starch (Thayumanavan and Sadasivam, 1984) and antioxidant like proline (Bates *et al*.,1973), lycopene (Zakaria *et al*., 1979), carotenoids (Bendich and Olson, 1989), total polyphenol (Eom *et al*., 2008), polyphenol oxidase (Esterbauer *et al*. 1991) and superoxide dismutase (Gong *et al.,* 2005) and Lipid peroxide (LPX) by the method of Ewa, 2006.

Results and Discussion

Fresh leaves of *Cyclea peltata* were studied for its nutritional and antioxidants properties and crude methanolic extract were used for the phytochemical investigation and *in vitro* anticancer activity analysis.

Phytochemicals

Phytochemicals are biologically active compounds, naturally occurring and non- essential nutrients. These compounds are major sources of a large number of medicines consisting of various groups like emetics, antispasmodics, antimicrobials, anti-cancerous etc. Till now, more than twelve thousand phytochemicals have been isolated from various plants (Ajuru *et al*., 2017). Phytochemical screening of *Cyclea peltata* revealed the presence of reducing sugar, flavonoids, tannins, steroids, alkaloids, terpenoids, steroids and saponins. But anthraquinones are not detected (Table 1).

Table 1. Preliminary Phytochemical Evaluation of *Cyclea peltata*

Sl. No.	Phytochemicals	Methanol Extract of *Cyclea peltata*
1	Reducing sugar	+++
2	Flavonoids	+++
3	Alkaloids	++
4	Tannins	++
5	Terpenoids	+
6	Steroids	+
7	Saponins	+
8	Anthraquinones	-

Quantitative Analysis

Nutritional Evaluation

Nutritional factors in *Cyclea peltata* were quantitatively analysed. The different nutritional factors like reducing sugar, total carbohydrate, total protein, pigments and starch were analysed. Sugars plays a vital role in plants both nutrient and central signalling or regulatory molecules that modulate gene expression related to plant growth, development, metabolism, stress response and disease resistance. Reducing sugar plays an important role inthe central metabolic pathways and help in the production of secondary metabolites that enhance the medicinal properties of plants (Deepa and Sumit, 2020). Reducing sugar from aerial parts of *C. peltata* were extracted and analysed by DNS method and the result found to be high (0.894 mg g-1) as show in figure 1. In plants, carbohydrates produced by photosynthesis are well known for their essential role as vital sources of energy and carbon skeletons for organic compounds and storage components. Carbohydrates play an important role in plant immunity (Trouvelot *et al*., 2014). The amount of total carbohydrate found in *C. peltata* was found to be higher (25.938 mg g -1) as shown in figure 2. Protein is made from twenty-plus basicbuilding blocks called amino acids. Proteins, highly complex substance that is present in all living organisms. Proteins are of great value and are directly involved in the chemical processesessential for life (Felix *et al*., 2019). The amount of total protein is found to be higher (28.918mg g-1) in *C. peltata* as shown in Fig. 2. Chlorophyll is the green pigment in plants, algae andcyanobacteria that is essential for photosynthesis. There are five forms of chlorophylls found in plants and photosynthetic organism, but in plant kingdom only two major forms are commonly found, that is, chlorophyll-a and b. These two forms of chlorophyll coexist in plantsin an approximate ratio of 3:1 with chlorophyll- a being predominant (Zhonghi *et al*., 2019). The amount of chlorophyll was estimated and found that high content of chlorophyll-a (0.819 mg g-1), chlorophyll-b (0.812 mg g-1) and total chlorophyll (1.236 mg g -1) were present in *C.peltata* as shown in Fig. 3. Starch is the most significant form of carbon reserve in plants. Starch is an insoluble, non-structural carbohydrate composed of α-glucose polymers (Martin *et al*., 1995). The amount of starch in *C. peltata* is (0.739 mg g-1) as shown in Fig. 4. The nutritionalanalysis of *C. peltata* showed the presence of high amount of reducing sugar, carbohydrate, protein, chlorophyll-a, chlorophyll-b, total chlorophyll and starch.

Evaluation of Antioxidant Properties

The aerial part of the plant material was analysed for its antioxidant properties. Both enzymatic and non-enzymatic antioxidants were evaluated. Non-enzymatic antioxidants like proline, lycopene, carotenoids and total polyphenol and enzymatic antioxidants like Superoxide dismutases (SOD), Poly Phenol Oxidase (PPO), amylase and Lipid Peroxidase (LPX) were estimated by standard estimation methods. Proline metabolism propels cellular signaling processes that promote cellular apoptosis or survival. Proline enhances abiotic/ bioticstress response will facilitate agricultural crop research and improve human health (Liang *et al*., 2013). The amount of proline in *C. peltata* is 0.499 mg g -1 and found to be higher as shown in figure 5. Lycopene is the red-coloured carotenoid predominantly found in tomato fruit, but in few other fruits or vegetables. It has claimed that it may alleviate chronic diseases such as cancers and coronary heart disease (Bramley and Peter, 2000). The amount of lycopene in methanol extract of *C. peltata* is 0.428 mg g-1 (figure 5). Carotenoids exist in abundance in fruitand vegetables and have been known to contribute to the body's defense against reactive oxygen species (Rao *et al*., 2007). The amount of carotenoids present in *C. peltata* is 0.416 mgg-1 and found to be high as shown in figure 5. Polyphenols are plant non-nutrient natural products or the so-called plant secondary metabolites found in fruits, vegetables and seeds that consumed daily. Polyphenols attained the prominent position due to their wide distribution in plant-based foods and significant evidence of negative correlation of their consumption with cancers, diabetes and cardiovascular diseases (Munawar *et al*., 2017). The result revealed thattotal polyphenol content in *C. peltata* is found to be 0.982 mg g -1 as shown in figure 5. Flavonoids are a type of secondary metabolites, the antioxidant effect of flavonoids is due to their hydroxyl groups, by scavenging free radicals or by chelating metal ions. The prevention of radical generation that damage the biomolecules leading to oxidative stress can be protectedby flavonoids and it also gives security against the many diseases such as cancer, cardiovascular and respiratory disorders, arthritis and early ageing (Sankhadip *et al*., 2018). The amount of total flavonoids in *C. peltata* is 0.918 mg g-1 and found to be high as shown in (figure 5). Superoxide dismutase (SOD) is one of the most effective components of the antioxidant defense system in plant cells against Reactive Oxygen Species (ROS) toxicity. Theenzyme can serve as an anti-inflammatory agent and can also prevent precancerous cell changes (Walid *et al*., 2018). The amount of superoxide dismutases in *C. peltata* is 1.899 mg g-1 and found to be high as shown in this experiment. Polyphenol oxidases (PPO) mediated reactions play important role in alteration of colour, flavor, texture and nutritional values of fruit and vegetable crops. PPO plays a major role in the development of brown pigments in plants. It is also responsible for the functions including defense, cell differentiation and somatic embryogenesis (Constabel and Barbehenn, 2008). The result revealed that the amount of enzymatic antioxidant polyphenol oxidase in *C. peltata* is 0.954 mg g-1. Amylases are enzymes which hydrolyze starch as reserve carbohydrate in plants and glycogen as reserve carbohydrate in animals into reducing fermentable sugars, mainly maltose and reducing limit dextrins (Kirti *et al*., 2015). The amount of enzymatic antioxidant amylase in *C. peltata* is 0.928 mg g-1 as shown in this experiment . Lipoperoxides is an enzyme found in a wide variety of organisms, from plants to humans to bacteria. Its function is to break down hydrogen peroxide (H_2O_2), which is one of the toxins produced as a by-product of using oxygen, for respiration (Ewa, 2006). The result revealed that the amount of enzymatic antioxidant lipoperoxide in *C. peltata*is 0.973 mg g-1.

Evaluation of Pharmacological Property

In vitro Anticancer Activity in Crude Methanol Extract of *Cyclea peltata*

Plants have been used for medical purposes since the beginning of human history and are the basis of modern medicine. Most chemotherapeutic drugs for cancer treatment are molecules identified and isolated from plants or their synthetic derivatives. Today, despite considerable efforts, cancer still remains an aggressive killer worldwide. Many compounds isolated from plants are being rigorously tested for their anticancer properties and that show specificity towards cancer cells. They can induce cell death and inhibit the growth of tumours(Michal *et al*., extract of *C. peltata* was evaluatedby using DLA and EAC cell lines and cell viability was determined by Trypan blue exclusionmethod. In this the viable cell suspension 1×10^6 cells in 0.1 ml was added to the tubes containing various concentrations (100, 500 and 1000 µg/ml) of test compounds and volume was made up to 1 ml using phosphate buffer saline (PBS). The mixtures were incubated for 3 hours at 37° C and were added with 2 drops of Trypan blue dye. Dead cell takes up the blue colour of Trypan blue while live cells do not take up the dye. Reduction in the viable cell countand increased nonviable cancer cell count towards normal in tumor host suggest antitumor effect against EAC (Ehrlich Ascites Carcinoma) and DLA (Dalton's lymphoma Ascites cells) cells in mice. Cyclophosphamide is used as standard anticancer compound. The results obtained from anticancer study revealed that the methanol extract of *C. peltata* leaves showedremarkable anticancer activity against both the test cell lines (DLA and EAC). Methanol extractof *C. peltata* showed 4.6, 70.9, 81.6% cytotoxicity in EAC compared to DLA which showed 39.9, 64.8, 57 76.1% cytotoxicity at the concentration of 100, 500 and 1000mg/ml. (figure 6.) theresult of anti-cancer study in DLA and EAC cell lines showed high activity with increasing concentration of the extract such as 100, 500 and 1000mg/ml of *C. peltata*. The result of present study demonstrated that *C. peltata* methanol extract have in vitro anticancer activity against DLA and EAC cell lines. Depending upon the concentration of the extract used, this plant exhibited moderate to highly potent anticancer activity. The plant extract was found to be effective against DLA induced solid tumour and EAC induced ascites tumour. This may be used to the development of effective therapeutic approaches towards the prevention or treatments of various types of cancer in human beings.

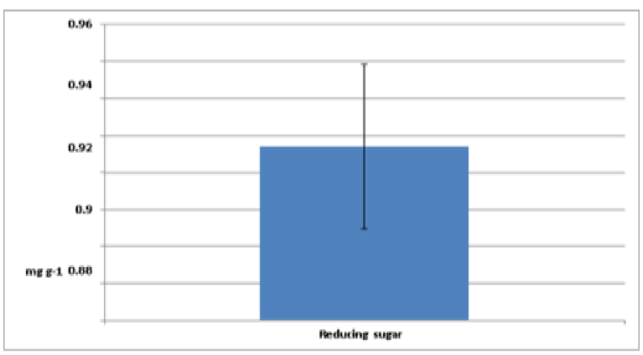

Figure 1. Reducing sugar in leaves of *C. peltata*

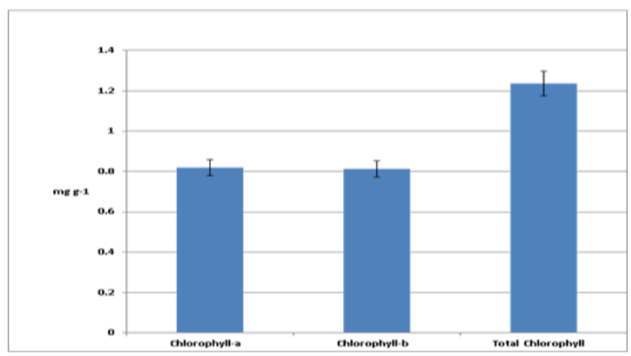

Figure 3. Pigments in leaves of *C. peltata*

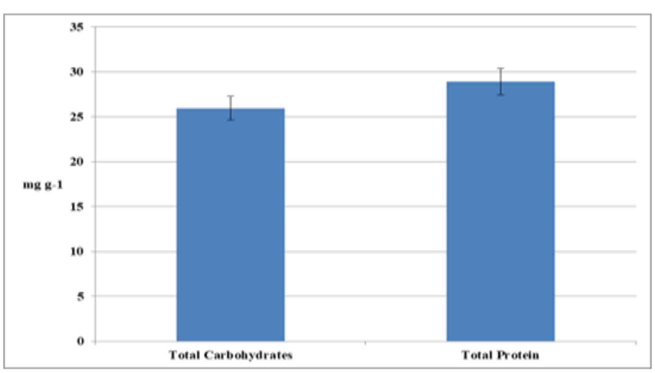

Figure 2. Total carbohydrates and Total protein in *C. peltata*

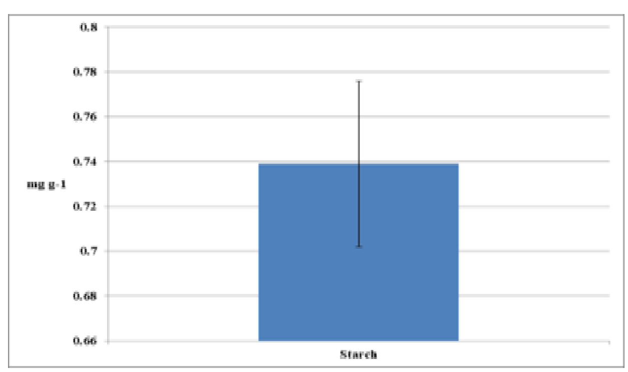

Figure 4. Starch in leaves of *C. peltata*

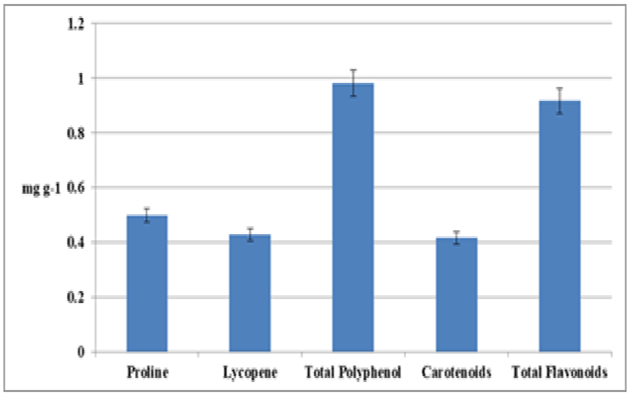

Figure 5. Non-Enzymatic Antioxidants in leaves of *C. peltata*

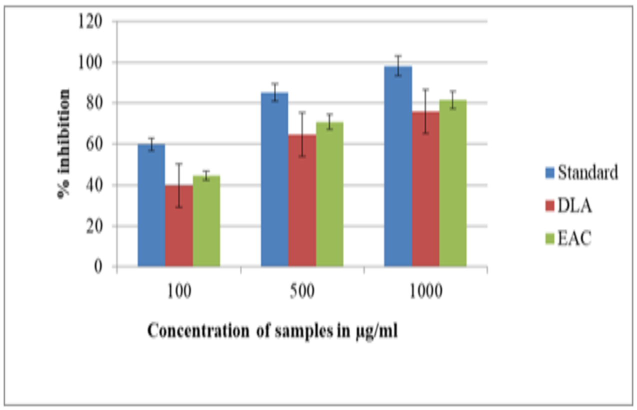

Figure 6. *In vitro* anticancer activity in leaves of *C. peltata*

Summary and conclusion

Present study revealed that *Cyclea peltata* has high amount of nutritional factors like reducing sugar, carbohydrates, protein, pigments and starch. The non-enzymatic and enzymatic antioxidants like proline, lycopene, total polyphenol, superoxide dismutase and polyphenol oxidase were also found to be higher. The plant extract was found to be effective against DLA induced solid tumour and EAC induced ascites tumour. This may be used to the development of effective therapeutic approaches towards the prevention or treatments of various types of cancer in human beings. The leaves *Cyclea peltata* has sufficient nutrients, antioxidants and invitro anticancer activity also. This generated information on phytochemical, nutritional, and medicinal characteristics and therapeutic potential of *Cyclea peltata* provide scientific proof for identifying the plant bio resource and its effective utilization in the future. In brief the wildspecies of *Cyclea peltata* fits its claims of nutritional and medicinal properties which satisfy its use as an ethnomedicinal plant by tribal communities of Kerala.

Acknowledgement

The authors are grateful to Dr. T. S. Swapna, Associate Professor, Department of Botany, University of Kerala, Kariyavattom, Thiruvananthapuram for necessary support and the Amala Cancer Hospital and Research Centre, Amalanagar, Thrissur for laboratory analysis.

Refference

Ajuru M.G., Williams L.F. and Anjuru G. (2017). Qualitative and quantitative phytochemical screening of some plants used in Ethnomedicine in the Niger delta region of Nigeria. *Journal of Food and Nutrition Science*. 5(5):198-205.

Balandrin M.F., Kjocke A.J., Wurtele E. Natural plant chemicals: sources of industrial and mechanical materials. *Science* 228: 1154-1160, 1985.

Bendich, A. D. and Olson, J. A. (1989). Biological actions of carotenoids 1. *The FASEBjournal*. 3(8): 1927-1932.

Bates L. S., Waldren R. P. and Teare I. D. (1973). Rapid determination of free proline forwater stress studies. *Plant Soil*. 39: 205- 207.

Benkeblia N. (2004) Antimicrobial activity of essential oil extracts of various onions (Allium cepa) and garlic (*Allium. sativum*). *Lebensm-Wiss u-Technology Journal* 37: 263-268.

Constabel C.P. and Barbehenn R. (2008). Defective roles of polyphenol oxidase in plants. J. *Intro Plant Resistance Herbi*. 8(12):253-270.

Colombo M.L., Bosisio E. (1996) Pharmacological activities of *Chelidonium. majus* L(Papaveraceae). *Pharmacological Research* 33: 127-134.

Deepa Khatri and Sumit B.B. (2020). Reducing sugar, total phenolic content and antioxidantpotential of Nepalese plants. *International Journal of Biomedical Research*. 10(55): 1-10.

Esterbauer H, Schwarzl E and Hayn M. (1977). A rapid assay for catechol oxidase and laccaseusing 2- nitro-5-thio benzoic acid. *Analatical Biochemistry*. 77: 486- 494.

Eom S.H., Jin C.W., Kim D.O., Seo D.W. and Jeong Y.H. (2008). Changes in antioxidant activity with temperature and time in *Chrysathemum indicum* L. teas during elution processesin hot water. *Food Science and Biotechnology*. 17: 408-412.

Gong H., Zhu X., Chen K., Wang S. and Zhang C. (2005). Silicon alleviates oxidative damageof wheat plants in pots under drought. *Plant Science*. 169: 313- 321.

Hedge J.E. and Hofrieter B.T. (1962). In: Methods in Carbohydrate Chemistry. (Eds. Whistler
R.L. and Be Miller J.N.). Academic Press, New York. 17: 420.

Iwu M.W., Duncan A.R. and Okunji C.O. (1999) New antimicrobials of plant origin. In: Janick
J. *et al*. Perspectives on New Crops and New Uses. Alexandria, VA: ASHS Press; pp. 457-462.

Krishnaraju A.V., Rao T.V.N. and Sundararaju D. (2005) Assessment of bioactivity of Indianmedicinal plants using Brine shrimp (*Artemia salina*) lethality assay. *International Journal of Applied Science and Engineering* 2: 125-134.

Lowry O.H., Rosebrough N.J., Farr A.L. and Randall R.J. (1951). Protein measurement with the folin phenol reagent. *Journal of Biological Chemistry*. 193:265-275.

Martins A.P., Salgueiro L., Goncalves M.J. (2001) Essential oil composition and antimicrobial activity of three Zingiberaceae from S. Tome e Principle. *Planta Med* 67: 580-584.

Rao, A. Venket, and Leticia G. Rao. (2007) "Carotenoids and human health." *Pharmacologicalresearch* 55.3: 207-216.

Trouvelot, Sophie, Marie-Claire Héloir, Benoît Poinssot, Adrien Gauthier, Franck Paris, Christelle Guillier, Maud Combier, Lucie Trdá, Xavier Daire, and Marielle Adrian. (2014): 69"Carbohydrates in plant immunity and plant protection: roles and potential application as foliar sprays." *Frontiers in plant science*: 592.

Tanaka H., Sato M., Fujiwara S. (2002). Antibacterial activity of isoflavonoids isolated from *Erythrina variegata* against methicillin resistant Staphylococcus aureus. *Letters in Applied Microbiology* 35: 494- 498.

Zakaria H., Simpson K., Brown P.R. and Krotulovic A. (1979). Use of reversed phase HPLC analysis for the determination of provitamin- A, carotenes in tomatoes. *Journal of Chromatography.* 176: 109-117.

Zohngli Pan., Zhang, R., and Zicari, S. (Eds.). (2019). Integrated Processing Technologies forFood and Agricultural By-Products. Academic press.1:1-19.