Original Research Article **Pharmacology**

International Journal of Pharma and Bio Sciences

ISSN 0975-6299

PHYTOCHEMICAL ANALYSIS OF LEAF EXTRACTS OF ANTIHYPERGLYCEMIC MEDICINAL PLANTS

KUMARI SMITA[1*], S. K. SARANGI[1], K. MANJUNATH[1]

[1]Department of Microbiology and Biotechnology, Bangalore University, JB campus, Bangalore , India

ABSTRACT

Primary screening of phytochemicals is a crucial step, in the detection of the bioactive compounds present in medicinal plants and consequently may lead to drug discovery and development. In the present study, important phytoconstituents of the selected medicinal plants *Ocimum canum* (*O. canum*) and *Costus igneus* (*C. igneus*), were reported in order to relate their presence with bioactivities of the plants. Three different solvents namely n-hexane, ethylacetate and methanol according to their increasing polarity were used for extraction of phytoconstituents from the leaf part of both the plants. Qualitative phytochemical analysis of leaf extract was performed for the presence of triterpenoids, tannins, glycosides, sterols, alkaloids, flavonoids, carbohydrates, proteins and organic acids. Further quantitative analysis was conducted to determine the amount of total phenolics, total flavonoids, total alkaloids and total antioxidant activity of the phytocostituents. There was significant difference between the presence and absence of different phytocompounds in various leaf extracts. The total phenolics content was found to be maximum in the methanolic extracts of both the plants. The total flavonoid content was found to be maximum in the hexane extract of *O. canum* and ethylacetate extract of *C. igneus.* Furthermore, the total antioxidant capacity of *O.canum* was found to be higher than *C. igneus*

KEYWORDS: ***Ocimum canum, Costus igneus; Secondary metabolites, Antihyperglycemic, Antioxidant, Phenolic, Flavonoid***

KUMARI SMITA*

Department of Microbiology and Biotechnology, Bangalore University, JB campus, Bangalore , India

Received on: 07-08-2017
Revised and Accepted on: 05-10-2017
DOI: http://dx.doi.org/10.22376/ijpbs.2017.8.4.p212-218

Creative commons version 4.0

INTRODUCTION

Medicinal Plants play a convincing role in the prevention and treatment of several diseases. They can be a reservoir of chemical compounds of biological and pharmacological importance. Previous reports suggest that plants are source of important drugs, and will progressively determining the screening of new lead compounds. The most important of these bioactive constituents of plants are flavonoids, phenolics, alkaloids and terpenoids compounds. The members of Lamiaceae family are used in traditional medicine for various disorders. Herbs belonging to this family such as basil, thyme oregano are used in culinary process throughout the globe. *Ocimum canum (O. canum)* belongs to family lamiaceae and earlier studies have shown that the aqueous extract of *O. canum* is used by Ghanaians to treat diabetes mellitus [1.] A number of phenolic compounds have been reported from the genus *Ocimum* [2]. *Costus igneus(C. igneus)* belongs to family Costaceae introduced from Central and South America. In southern India, the leaves are used as a dietary supplement in the treatment of diabetes mellitus [3.] Extracts of herbs, vegetables, cereals, and other plant materials rich in phenolics, flavonoids and triterpenoids are of interest in food industry because they reduce oxidative degradation of lipids and thereby improving the quality and nutritional value of food. Ingestion of diet rich in plant polyphenols and flavonoids offers protection against development of diabetes and other epidemic diseases [4- 6]. Flavonoids are low molecular weight bioactive phenols and ubiquitously present in fruit, vegetables, flower and seeds [7-8]. The flavonoids play an important role in fighting against the complication of diabetes [9]. Quercetin is well known natural flavonoid present in plants kingdom. It showed antioxidant effect on experimental streptozotocin (STZ)-induced diabetes in rats [10]. Hespiridin, the other flavonoid, is attenuated with diabetic complications and also reverses neuoropathic pain by reducing blood sugar and lipid to down-regulate generation of free radicals and release of proinflammatory cytokines [11]. Alkaloids affect central nervous system, reduce appetite and behave as diuretic [12]. The bioactive components of medicinal plants have been considered as a new approach in the prevention and as well as the management of diabetes. This study aimed to investigate and characterize the bioactive compounds in the different leaf extracts of *O. canum* and *C.igneus.*

MATERIALS AND METHODS

Materials

The chemical reagent DPPH (2,2- diphenyl 1,1-picrylhydrazyl) and Trolox (6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) was purchased from Sigma-aldrich (Bangalore, India) all other chemicals used were analytical grade and obtained from SISCO RESERCH LAB. Pvt. Ltd (Mumbai, India).

Collection and preparation of plant material

Seeds of *O. canum* were collected, identified and authenticated from the Institute of Integrated Ayurveda and Medicine, Bangalore. The leaves of each plant were harvested at full bloom, weighed, bulked and placed in a paper bag kept for air dry at room temperature for 15 days. *C .igneus* leaves were collected washed and oven dried at 35º C for 2 days. Leaf sample was crushed using mixer to fine powder. The powder was homogenized with liquid nitrogen using pestle and mortar. Three different solvents with increasing polarity such as n-hexane, ethyl acetate and methanol were used for the successive extraction process. Samples were centrifuged (REMI-24BL) at 6000 rpm at 4º C for 10 min. Supernatant was collected and evaporated.. Prepared leaf extract was filtered and kept in -20ºC for further analysis. [13]

Qualitative Phytochemical tests

Qualitative analysis of phytoconstituents of the leaf extracts were carried out with the standard methods [14].

Test for triterpenoids

Salkowsaki test **:** Leaf extract (5 ml) was mixed with 2 ml of chloroform, and 3 ml of concentrated sulphuric acid was added slowly to the side of test tube and allowed to form a layer. A reddish brown color of the interface indicated presence of triterpenoids.

Test for Tannins

5% Ferric chloride was added to 5 ml leaf extract and allowed to stand. Formation of a deep blue black color indicated the presence of tannins.

Test for saponin

Foam test: Leaf extract (2 ml) was mixed with water and shaken vigorously. This lead to the formation of froth which was stable for 5 minutes. This indicated the presence of saponin.

Test for glycosides

Bromine water test**:** Bromine water was added to different leaf extracts. The formation of a yellow precipitate indicated the presence of glycosides.

Test for sterols

Salkowaski test **:** In 2ml of leaf extract, 2ml of chloroform and 2ml of concentrated sulphuric acid was added slowly and shaken well. The chloroform layer turned red and sulphuric acid layer turned greenish yellow, which indicated the presence of sterols.

Test for Alkaloids

The leaf extract (5 ml) was acidified by adding 2 ml of diluted hydrochloric acid .It was shaken well and filtered. The filtrate was used for the analysis.

Dragendroff's Test

1 ml of Dragendroff's reagent when added to the acidified filtrate, gave orange precipitate immediately. This indicated the presence of alkaloids in the test sample**.**

Test for flavonoids

Ferric chloride test**:** Few drops of ferric chloride were added to the 5 ml of leaf extract. The formation of an intense green color indicated the presence of flavonoids.

Test for carbohydrates

Few drops of Molisch reagent were added to 5 ml of leaf extract followed by addition of concentrated sulphuric

acid slowly along the wall of the test tube. The formation of a violet ring at the junction of two liquids indicated the presence of carbohydrates.

Test for proteins

Biuret test**:** Leaf extract (5 ml) was treated with copper sulphate solution. Formation of a violet color complex indicated the presence of proteins in the test sample.

Test for organic acids

Few drops of calcium chloride were added to the 5 ml of leaf extract. Immediate precipitation was observed which indicated the presence of organic acids.

Quantitative Phytochemical analysis

Quantitative analysis of phytoconstituents of the leaf extract (2 mg/ml in DMSO) was carried out using standard methods.

Measurement of total phenolic content

The total phenolic content (TPC) of the leaf extracts was determined with the Folin-Cio-calteu reagent by the method described by Bhoyar *et.al.,*(2011) [15]. Aliquots of standard gallic acid (200µg/ml) ranging from 10-100µl was pipetted into different test tubes. The volume in each test tube was made up to 500µl by 80% methanol. To each tube 2.5ml of FC reagent (1:1) followed by 2ml of 7.5% sodium carbonate was added. The tubes were incubated in dark for one hour. The absorbance was measured at 765nm by UV-1800 (Shimadzu) spectrophotometer. 500µl of leaf extract of different solvents methanol, ethyl acetate and hexane were taken as a sample. Results were expressed as of gallic acid equivalent (mg GAE/g).

Measurement of total Flavonoids content

Aluminum chloride colorimetric method was used for determination of total flavonoids as described by Chang *et.al.,*(2002) [16]. Aliquots of standard quercetin (10µg/ml) was taken ranging from 100-500µl in the test tubes, and made up to 500µl with 80% methanol followed by 1.5ml of 95% of methanol, then 0.1ml AlCl3(10%), 0.1ml Na-K tartrate and 2.8 ml distilled water was added sequentially. The test solution was vigorously shaken. The absorbance at 415 nm was recorded by using UV-1800 (Shimadzu) spectrophotometer after 30 minutes of incubation. Results were expressed as of quercetin equivalent (mg/g). 500µl of leaf extract of different solvents methanol, ethyl acetate and hexane were taken as a sample.

Measurement of total alkaloid content

Total alkaloid content was estimated by BCG method as described by Fazel. *et.al.,*(2008) [17] method. Aliquots of standard Atropine sulphate (10 mg/ml) was taken ranging from 100-500µl and made up to 500µl with phosphate buffer in test tubes then transfer each to different separating funnels. 5 ml of phosphate buffer (pH 4.7) along with 5 ml of BCG solution were added to each separating funnel and shaken vigorously with 1 to 5 ml of chloroform subsequently. The mixture were collected in 10 ml volumetric flasks and then diluted to adjust volume up to the mark with chloroform. Now, the absorbance of the complex in chloroform was measured at 470 nm against the blank. The leaf extract was acidified by adding diluted HCL. It was shaken well and and filtered. The 500µl of leaf of filtrate was used as a sample for the analysis.

Measurement of total antioxidant activity

The trolox equivalent antioxidant capacity (TEAC) was estimated by the method described by Sanchez et.al.,1998 [18]. Different concentration of standard trolox (0.40 mM/ml) ranging from 0.1 – 1.0 ml were pipetted out into test tubes and the volume was made up to 1 ml with 80% methanol. Then 3 ml of DPPH was added and was incubated in dark for 30 min at room temperature. The absorbance was measured at 517nm by UV-1800 (Shimadzu) spectrophotometer and percent scavenging was calculated. Different aliquots ranging from 100µg/ml to 1000µg/ml of leaf extract were used as sample. The antioxidant capacities of samples, were measured against trolox standard and expressed as TEAC.

STATISTICAL ANALYSIS

Three replicates of each sample were used for statistical analysis. Mean and standard deviations for all the analysis were calculated. Graphs were prepared with Graphpad Prism 7.00.

RESULTS AND DISCUSSION

Qualitative and quantitative analysis was performed to investigate the phytoconstituents of different leaf extracts of *O. canum and C. igneus.* The findings reveal the presence and quantity of various bioactive secondary metabolites which might be responsible for the medicinal property of these plants. The observations and inferences made in the phytochemicals tests are presented as follows.

Yield of leaf extracts

With the three different solvents, the percent yield of leaf extract in both the plants varied significantly. The yield of leaf extract in *O. canum* was found to be the highest with the methanol followed by ethyl acetate and hexane. In *C. igneus,* the percent yield of leaf extract with methanol was found to be the highest followed by hexane and ethyl acetate. The most important parameter is the solvent polarity in which higher the polarity, better is the solubility of phenolic compounds. The highest extract yield (up to 22.8%) was obtained with polar alcohol based solvents. [19]. Present results with a higher yield of leaf extracts in methanol in both the plants, are in agreement with the earlier report (Fig.1).

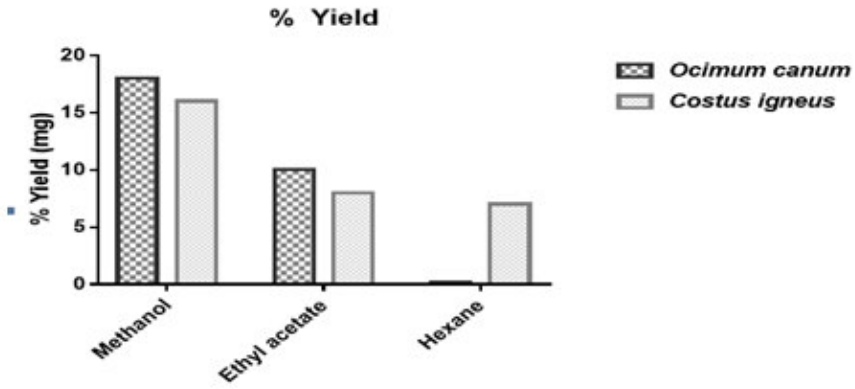


Figure 1
Changes in the percentage yield of Ocimum canum and Costus igneus

Qualitative phytochemical analysis of leaf extracts

With the three different solvents, the presence and absence of various compounds in both the plants varied significantly as described in Table (1, 2). Terpenoids, tannins, saponin, glycosides, alkaloids, flavonoids, carbohydrates, proteins and organic acid were found to be present in methanolic extract of *O.canum.* Tannins, saponin, glycosides, flavonoids and carbohydrate were found to be present in ethyl acetate extract whereas, tannins, saponin, glycosides, sterols, flavonoids and carbohydrates were also found to be present in the hexane extract. Saponin, glycosides, alkaloids, flavonoids, carbohydrates, protein and organic acids were found to be present in the methanolic extract of *C.igneus.* Tannin, saponin, glycosides, sterols, alkaloids, flavonoids and protein were found to be present in ethylacetate extract whereas, saponin, glycosides, sterols, flavonoids, carbohydrate and organic acids were present in hexane extract. Secondary metabolites attributes towards the bioactivities of medicinal plants such as hypoglycemic, antioxidant, anti-inflammatory and anti-carcinogenic properties [20].

Table 1
Qualitative phytochemical analysis of leaf extracts of Ocimum canum

Sl. No.	Qualitative Tests	Methanol	Ethyl acetate	Hexane
1	Triterpenoids	+	-	-
2	Tannins	+	+	+
3	Saponin	+	+	+
4	Glycosides	+	+	+
5	Sterols	-	-	+
6	Alkaloids	+	-	-
7	Flavonoids	+	+	+
8	Carbohydrates	+	+	+
9	Proteins	+	-	-
10	Organic acids	+	-	-

Table 2
Qualitative phytochemical analysis of leaf extracts of Costus igneus

Sl. No.	Qualitative Tests	Methanol	Ethyl acetate	Hexane
1	Triterpenoids	-	-	-
2	Tannins	-	+	-
3	Saponin	+	+	+
4	Glycosides	+	+	+
5	Sterols	-	+	+
6	Alkaloids	+	+	-
7	Flavonoids	+	+	+
8	Carbohydrates	+	+	+
9	Proteins	+	+	-
10	Organic acids	+	+	+

Total phenolic content in the leaf extracts

The maximum value of total phenolic content in *O. canum* and *C. igneus* was found to be 17.78 ± 0.30 mg/g and 6.24± 0.029 mg/g gallic acid equivalent respectively in the methanolic extracts. The results showed a significant difference in phenolic content with different solvents in both the plants as shown in Table (3) and Fig. (2). Phenolic acid has been reported in an extensive range of vegetables, fruits, and medicinal plants which is repeatedly implicated as natural antioxidants [21]. Phenolic compounds show redox properties which can play important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides [22]. Rosamarinic acid, a polyphenol which was reported in *Ocimum* genus has been found to be a potent antioxidant [23] and it has inhibitory activity on one of the important target carbohydrate hydrolyzing enzyme such as pancreatic alpha amylase [24]. Phenolic substances have been reported to have multiple biological effects, including antioxidant activity.

Table 3
Total Phenolics content in different solvents of leaf extracts of Ocimum canum and Costus igneus .

Sl. No.	Solvents	*Ocimum canum* (mg/g) Mean ± SD	*Costus igneus* (mg/g) Mean ± SD
1	Methanol	17.78 ± 0.30	6.24± 0.029
2	Ethyl acetate	15.58 ± 0.28	1.46 ± 0.005
3	Hexane	10.4 ± 0.17	5.65 ± 0.083

Values are representatives of mean ±S.D (n=3), Data of total phenolics are expressed as milligrams of gallic acid (GAE) equivalents per gram of dry weight.

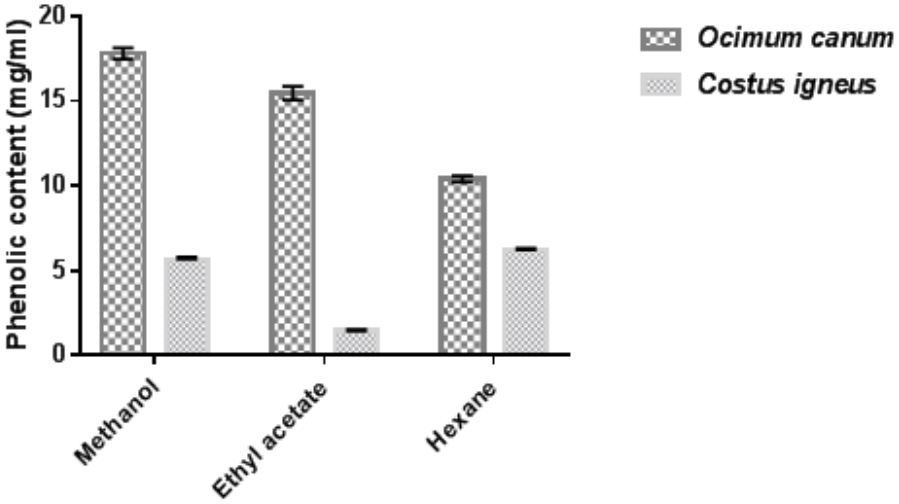


Figure 2
Changes in the total phenolic content of Ocimum canum and Costus igneus

Total flavonoid content in the leaf extracts

In *O. canum* leaf extract, the total flavonoid content was found to be maximum of 9.04 ± 1.96 mg/g of quercetin equivalent with hexane whereas, in *C. igneus* leaf extract the total flavonoid content was found to be maximum of 3.42 ± 0.06 mg/g quercetin equivalent with ethyl acetate. The total flavonoid content was found to be varied with three different solvents in both *O. canum* and *C. igneus* leaf extract as shown in Table (4) and Fig. (3). The total flavonoid content was found to be higher in the *O. canum* leaf extract than *C. igneus*. Flavonoids represent the most common and widely distributed groups of plant phenolics. It acts as an antioxidant that functions in scavenging free radicals, inhibiting lipid peroxidation and chelating transition metals. *C. igneus* has proven hypoglycemic effect. The hypoglycemic effects is attributed by the presence of several components like triterpene saponins, individual triterpene acids, flavonoids and other plant derived compounds which are present in the methanolic extract of *C. igneus* [25.] Antidiabetic activity might be attributed due to the presence of pentacyclic triterpene compound such as β- amyrin and β- L- Arabinopyranose methyl glucoside. [26].

Table 4
Total Flavonoid content in different solvents of leaf extracts of Ocimum canum and Costus igneus

Sl. No.	Solvents	*Ocimum canum* (mg/g) Mean ± SD	*Costus igneus* (mg/g) Mean ± SD
1	Methanol	8.68 ±1.04	2.17 ± 0.93
2	Ethyl acetate	6.48 ± 0.63	3.42 ± 0.06
3	Hexane	9.04 ± 1.96	1.2 6± 0.77

Values are representatives of mean ±S.D (n=3), Data of total flavonoids are expressed as milligrams of quercetin (QE) equivalents per gram of dry weight

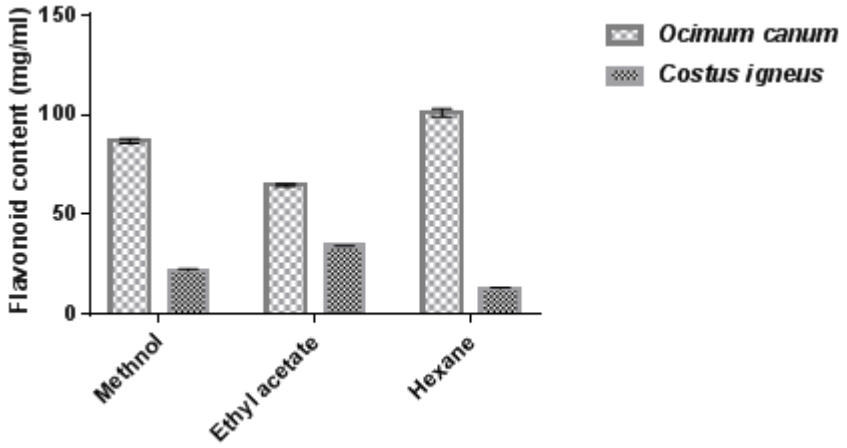


Figure 3
Changes in the total flavonoid content of Ocimum canum and Costus igneus

Total alkaloid content in the leaf extracts
There was significant difference in the total alkaloid content of both the plants. The total alkaloid content was found to be 0.76 ± 0.20 mg/g of atropine equivalent in *O. canum* methanolic leaf extracts whereas, it was found to be 2.24± 0.16 mg/g atropine equivalent in methanolic extract of *C. igneus*. Total alkaloid content was found to be quite higher in *C. igneus* leaf extract than *O. canum* . The antihyperglycemic property of *C. igneus* leaf extract may be attributed by the total alkaloid content.

Total antioxidant activity (TAA) of the leaf extracts
Total antioxidant activity of *O. canum* in methanolic extract was 60 µM/g of trolox equivalent whereas, in case of *C. igneus* TAA was found to be 10 µM/g. The TAA was found to be significantly higher of *O. canum* compare to *C. igneus.* The TAA results compared favorably with previous studies on Iranian *Ocimum* accessions extract in acetone which ranged from 10.8 to 35.7 µM/g of trolox equivalent [27]. Free radicals contribute to the progression of diabetic complications due to their ability to damage lipid, protein and DNA [28]Reactive oxygen species also develop insulin resistance which is one of the major causes of diabetes II[29]. Plant based antioxidants may be useful for the treatment free radical induced diseases like diabetes. On the basis of current knowledge of pathophysiology of insulin resistance, multiple approaches have been developed. The bioactive components of common plants have been considered as a new approach in the prevention and as well as the management of diabetes.

CONCLUSION

The emergence of diabetes related disorders, and the undesirable side-effects of currently used anti-diabetic drugs have motivated researchers in recent times to identify and investigate new alternative drugs from natural resources. Leaves of *O. canum* and *C.igneus* have traditionally been used in the treatment of hyperglycemia. The medicinal role of these plants could be related to the presence of bioactive compounds as identified and quantified in this paper. Further research and efforts to utilize the presence of phytocompounds in these medicinal plants hold a great potential to advance their biomedical application in the treatment of diabetes related disorders.

ACKNOWLEDGEMENT

I offer my sincere gratitude to the Council of Scientific and Industrial Research (CSIR), Government of India, for financial support in the form of Junior Research Fellowship during this research work.

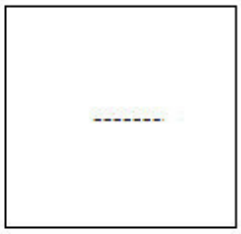
CONFLICT OF INTEREST

Conflict of interest declared none.

REFERENCES

1. Nyarko AK, Asare-Anane H, Ofosuhene M, Addy ME, Teye K, Addo P. Aqueous extract of Ocimum canum decreases levels of fasting blood glucose and free radicals and increases antiatherogenic lipid levels in mice. Vascular pharmacology. 2002 Dec 31;39(6):273-9.
2. Nakatani N, Antioxidants from spices and herbs. In: Shahidi F, editor. Natural antioxidants: chemistry, health effects, and applications. Champaign, IL: AOCS Press; 1997. p. 64–75.
3. Shetty AJ, Choudhury D, Rejeesh VN, Kuruvilla M, Kotian S. Effect of the insulin plant (Costus igneus) leaves on dexamethasone-induced hyperglycemia. International journal of Ayurveda research. 2010 Apr;1(2):100.
4. Arts IC, Hollman PC. Polyphenols and disease risk in epidemiologic studies. The American journal of clinical nutrition. 2005 Jan 1;81(1):317S-25S
5. Graf BA, Milbury PE, Blumberg JB. Flavonols, flavones, flavanones, and human health: epidemiological evidence. Journal of medicinal food. 2005 Sep 1;8(3):281-90.
6. Pandey KB, Rizvi SI. Plant polyphenols as dietary antioxidants in human health and disease. Oxidative medicine and cellular longevity. 2009;2(5):270-8.
7. Heim KE, Tagliaferro AR, Bobilya DJ. Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships. The Journal of nutritional biochemistry. 2002 Oct 31;13(10):572-84.
8. Hollman PH, Katan MB. Dietary flavonoids: intake, health effects and bioavailability. Food and Chemical Toxicology. 1999 Oct 31;37(9):937-42.
9. Gomathi D, Ravikumar G, Kalaiselvi M, Devaki K, Uma C. Effect of Evolvulus Alsinoides Extract on Membrane Bound Enzyme Activities in the Tissues of Streptozotocin Induced Diabetic Rat. Asian Journal of Pharmaceutical Research and Health Care. 2014 Apr 3;6(1):1-5.
10. Abdelmoaty MA, Ibrahim MA, Ahmed NS, Abdelaziz MA. Confirmatory studies on the antioxidant and antidiabetic effect of quercetin in rats. Indian Journal of Clinical Biochemistry. 2010 Apr 1;25(2):188-92.
11. Shi X, Liao S, Mi H, Guo C, Qi D, Li F, Zhang C, Yang Z. Hesperidin prevents retinal and plasma abnormalities in streptozotocin-induced diabetic rats. Molecules. 2012 Nov 1;17(11):12868-81
12. United States Department of Agriculture, Center for Nutrition Policy and Promotion. Dietary Guidelines for Americans. National Academy Press, Washington DC: USA; 2010. Available from: https://www.cnpp.usda.gov/dietary-guidelines
13. Amoo SO, Ndhlala AR, Finnie JF, Van Staden J. Antibacterial, antifungal and anti-inflammatory properties of Burchellia bubalina. South African Journal of Botany. 2009 Jan 31;75(1):60-3.
14. Harborne JB. Phytochemical methods, London. Chapman and Hall, Ltd. London: U.K., 1973. p. 49-188.
15. Bhoyar MS, Mishra GP, Naik PK, Srivastava RB. Estimation of Antioxidant Activity and Total Phenolics Among Natural Populations of Caper ('Capparis spinosa') Leaves Collected from Cold Arid Desert of Trans-Himalayas. Australian Journal of Crop Science. 2011 Jul;5(7):912.
16. Chang CC, Yang MH, Wen HM, Chern JC. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. Journal of food and drug analysis. 2002 Jul 1;10(3).
17. Fazel S, Hamidreza M, Rouhollah G, Verdian-rizi M. Spectrophotometric determination of total alkaloids in some Iranian medicinal plants. THE SOCIETY FOR ADVANCEMENT OF HORTICULTURE. 2010 Jan;12(1):69-70.
18. Sánchez-Moreno C, Larrauri JA, Saura-Calixto F. A procedure to measure the antiradical efficiency of polyphenols. Journal of the Science of Food and Agriculture. 1998 Feb 1;76(2):270-6.
19. Tomsone L, Kruma Z, Galoburda R. Comparison of different solvents and extraction methods for isolation of phenolic compounds from horseradish roots (Armoracia rusticana). World Academy of Science, Engineering and Technology. 2012 Apr 29;64:903-8.
20. Negi JS, Singh P, Rawat B. Chemical constituents and biological importance of Swertia: a review. Curr Res Chem. 2011;3(1):1-5.
21. Velioglu YS, Mazza G, Gao L, Oomah BD. Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. Journal of agricultural and food chemistry. 1998 Oct 19;46(10):4113-7.
22. Osawa TO. Novel natural antioxidants for utilization in food and biological systems. Postharvest biochemistry of plant food-materials in the tropics. 1994:241-51.
23. Berhow MA, Affum AO, Gyan BA. Rosmarinic acid content in antidiabetic aqueous extract of Ocimum canum Sims grown in Ghana. Journal of medicinal food. 2012 Jul 1;15(7):611-20.
24. McCue PP, Shetty K. Inhibitory effects of rosmarinic acid extracts on porcine pancreatic amylase in vitro. Asia Pacific Journal of Clinical Nutrition. 2004 Mar 1;13(1).
25. Krishnan KR, Vijayalekshmi NR, Helen A. Methanolic extract of Costus igneus (NE Br) alleviates dyslipidemia in diabetic rats. Asian J Pharm Clin Res. 2011;4:154-7.
26. Aruna A, Nandhini SR, Karthikeyan V, Bose P, Vijayalakshmi K. Comparative anti-Diabetic effect of Methanolic extract of Insulin Plant (Costus pictus) leaves and its silver nanoparticle. Indo American Journal of Pharmaceutical Research. 2014;4(7):3217-30.
27. Javanmardi J, Stushnoff C, Locke E, Vivanco JM. Antioxidant activity and total phenolic content of Iranian Ocimum accessions. Food chemistry. 2003 Dec 31;83(4):547-50.
28. Ayepola OR, Brooks NL and Oguntibeju OO. Oxidative stress and diabetic complications: the role of antioxidant vitamins and flavonoids in antioxidant-antidiabetic agents and human health InTech, 2014.
29. Erejuwa OO. Oxidative stress in diabetes mellitus: is there a role for hypoglycemic drugs and/or antioxidants?. InOxidative stress and Diseases 2012. InTech.

Reviewers of this article



Dr.V.Veeramanickandan Ph.D

Assistant Professor
Research Department of Microbiology
MGR College Hosur-635130

Prof.Dr. M.Ranga Priya, M.Pharm., Ph.D., R.Ph.

Professor, Dept of Pharmaceutics,Sun Institute Of Pharmaceutical Education & Research, Kakupall, Nellore Rural, Nellore, Andhra Pradesh 524346

Prof.Dr.K.Suriaprabha

Asst. Editor , International Journal of Pharma and Bio sciences.

Prof.P.Muthuprasanna

Managing Editor , International Journal of Pharma and Bio sciences.

We sincerely thank the above reviewers for peer reviewing the manuscript