Journal of Pharmaceutical Research International

33(59B): 437-445, 2021; Article no.JPRI.79887
ISSN: 2456-9119
(Past name: British Journal of Pharmaceutical Research, Past ISSN: 2231-2919, NLM ID: 101631759)

Phytochemical Screening and *In vitro* Albumin Denaturation Inhibitory Potential of Methanolic Root Extract of *Acorus calamus*

M. Sanjay Varshan [a], R. Gayathri [a*], V. Vishnu Priya [a], J. Selvaraj [a] and S. Kavitha [a]

[a] Department of Biochemistry, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai-600 077, India.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2021/v33i59B34401

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/79887

Original Research Article

Received 08 October 2021
Accepted 17 December 2021
Published 18 December 2021

ABSTRACT

Introduction: Medicinal plants are chief antidotes for numerous diseases and have been used since time immemorial. Sweet flag's (*Acorus calamus*) presence is in Ayurveda and belongs to the genus Acorus L. of the family Acoraceae and is widely distributed in temperate to sub temperate regions. It is commonly used to treat appetite loss, diarrhoea, digestive disorders in traditional medicinal systems of Asian and European countries. The aim of this study is to explore the phytoconstituents, antioxidant and anti-inflammatory potential of methanolic root extract of *Acorus calamus.*

Materials and Methods: Methanolic root extract of *Acorus calamus* was done by the Hot Percolation method. Later it was dried and used to analyse the antioxidant and anti-inflammatory potentials. Phytochemical screening was done to analyse the presence of various phytochemicals. Antioxidant effect of *Acorus calamus* was tested by 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity and Albumin denaturation inhibitory potential test was organised for testing it's anti-inflammatory Activity. The data were analysed statistically by a one-way analysis of Variance (ANOVA) followed by Duncan's multiple range test was used to see the statistical significance among the groups. The results with the $p<0.05$ level were considered to be statistically significant.

**Corresponding author: E-mail: gayathri.sdc@saveetha.com;*

Results: Methanolic root extract of *Acorus calamus* was found to be rich in Alkaloids, Flavonoids, Terpenoids, Sapanoids, Steroids and Phlobatannin. The presence of phytochemicals like alkaloids, saponins, Flavonoids indicates that the extract has potential for further in vitro analysis like antioxidant and anti-inflammatory potentials. It was observed that *Acorus calamus* has both antioxidant (IC_{50} of = 295 µg/ml) as well as anti inflammatory potentials (IC_{50} =310 µg/ml) and the activity increased in a dose dependent manner as compared to that of standard (Vitamin C and Diclofenac respectively).
Conclusion: The study proves the antioxidant and anti-inflammatory efficacy of *Acorus calamus* and throws light on the prospects of drug formulation against oxidant activity and inflammation.

Keywords: Anti inflammation; antioxidant; Acorus calamus; diclofenac; DPPH; albumin denaturation inhibition assay; innovative technology; novel method.

1. INTRODUCTION

Nearly 80% of the world's population in developing countries mainly depends on natural products for their health needs [1]. Mother Earth has blessed mankind with various plants with healing ability for curing the ailments of human beings. The WHO has also estimated that 80% of the world's population meets their primary health care needs by the traditional medicines which came through their ancestors [2]. Plant products are used as the main source of medicine throughout the world for treating various human ailments [3].

Acorus calamus Linn., commonly known as sweet flag, belongs to the family Acoraceae, which is commonly found in India. In Ayurvedic medicine, calamus is an important herb, valued as a 'rejuvenator' for the nervous system [4]. The rhizome of calamus is used for various medicinal purposes mainly appetite loss, fever, stomach cramps. It is a semi aquatic perennial aromatic herb [5]. The parts of the plant *Acorus calamus* generally used are leaves, root (rhizome) and stem. In Ayurvedic medicinal practice, the rhizomes have been used to cure several diseases like asthma, bronchitis and also acts as a sedative [6]. Native tribes used it to treat coughs, digestive problems such as gas, bloating and poor digestive functions. Sweet flag is also used externally to treat skin eruptions, rheumatic pains and neuralgia [7].

The main chemical constituents of this plant are Asanone, Calamenol, Calamine, eugenol, Camphene, various fatty acids, sugars and glycosides [8]. These are bioactive chemicals of plant origin, which are considered as secondary metabolites [9]. The successful determination of active principles isolated from plant material is predominantly dependent on the variety of solvent used in extraction methods [10]. Hence, it emphasises that numerous solvent extractions are required to screen the plant parts for phytochemicals.

Many antioxidant compounds can be found in fruits and vegetables including phenolic, carotenoids, anthocyanins and tocopherols. Approximately 20% of known plants have been in use in pharmaceutical studies, affecting the healthcare system in positive ways such as treating cancer and harmful diseases [11]. Phenolic compounds found in plants have been well known for their ability of scavenging free radicals, which is referred to as antioxidant potential [12].

Free radicals have a significant role in processes of chemical materials degradation and also contribute to more human disorders [13]. Antioxidants are widely used in dietary supplements and have been investigated for prevention of diseases such as cancer and inflammatory disorders. These also give protection to living organisms from damage caused by uncontrolled production of oxidative stress, concomitant lipid peroxidation and protein damage [14].

Inflammation has been associated with many diseases which bring about hazardous effects on patients sometimes causing a threat to their lives. There are many anti inflammatory drugs found recently to treat consequences of inflammation belonging to steroidal or non steroidal anti inflammatory drugs (NSAIDS), but these can also cause acute renal failure [15]. For these reasons, many researchers have shifted their focus on finding medicinal plants, which are rich in anti inflammatory property and can serve as a potential ingredient for future drug development [16-18].

Many studies have been conducted on plants to investigate their antioxidant and anti-inflammatory potentials and also these plants have been screened for phytochemicals, which are actively associated with its above-mentioned properties [19]. Over the years, one of the most popular methods to study in vitro anti-inflammatory properties of plant extract is done by Albumin denaturation inhibition assay and antioxidant potential by DPPH assay [20-21]. Our team has extensive knowledge and research experience that has translate into high quality publications [22-36]. The main aim of this study is to assess the phytoconstituents, antioxidant and in vitro anti denaturation potential of methanolic root extract of *Acorus calamus*.

2. MATERIALS AND METHODS

2.1 Plant Extract

Acorus calamus was purchased from an Ayurvedic farm. Air dried, crushed and made into powder form. Methanol was added.80% of methanolic extract was obtained. The extract was prepared by a hot percolation method. Later it was dried and used to analyse the antioxidant and anti-inflammatory potential.

2.2 Phytochemical Screening Test

2.2.1 Test for Phlobatannin

1ml of the extract was treated with 1ml of 1% HCl and boiled for 10 mins. The formation of red color precipitate indicates the presence of phlobatannin.

2.2.2 Test for carbohydrates

Three to five drops of Molisch reagent was added with 1 mL of the extract and then 1 mL of concentrated sulphuric acid was added carefully through the side of the test tube. The mixture was then allowed to stand for two minutes and diluted with 5 mL of distilled water. The development of a red or dull violet ring at the junction of the liquids showed the presence of carbohydrates.

2.2.3 Test for flavonoids

Few drops of 1% liquid ammonia were taken in a test tube and along with it 1ml of the extract was added resulting in the formation of yellow color thereby indicating the presence of flavonoids.

2.2.4 Test for alkaloids

2ml of sample was mixed with 2ml of HCl. Then 6 drops of HCN was added and further 2 drops of picric acid was added that resulted in a creamish pale yellow ppt indicating the presence of alkaloids.

2.2.5 Test for terpenoids

2 ml of sample along with 2ml of chloroform and 3ml of con. H2SO4 was added. Red color ppt obtained indicates the presence of terpenoids.

2.2.6 Test for proteins

One milliliter of ninhydrin was dissolved in 1 mL of acetone and then a small amount of extract was added with ninhydrin. The formation of purple colour revealed the presence of protein.

2.2.7 Detection of saponins

Foam test: A fraction of the extract was vigorously shaken with water and observed for persistent foam.

2.2.8 Test for steroids

One milliliter of chloroform was mixed with 1 mL of extract and then ten drops of acetic anhydride and five drops of concentrated sulphuric acid were added and mixed. The formation of dark red colour or dark pink colour indicates the presence of steroids.

2.2.9 DPPH free radical scavenging activity

Scavenging of 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radicals was assessed by the method of Hatano et al, (1989). DPPH solution (1.0 ml) was added to 1.0 ml of the root extract at different concentrations (0.1 to 0. 5mg/ml). The mixture was kept at room temperature for 50 minutes and the activity was measured at 517 nm. Ascorbic acid was used as standard in this experiment at the same concentrations. The capability to scavenge the DPPH radical was calculated and expressed in percentage (%) using following formula:

$$\text{DPPH radical scavenging (\%)} = \frac{(\text{Control OD} - \text{Sample OD}) \text{ X } 100}{\text{Control OD}}$$

In vitro anti-inflammatory activity of methanolic root extract of *Acorus calamus* by albumin denaturation inhibition assay: The anti-inflammatory activity of methanolic root extract of *Acorus calamus* was studied by using inhibition of albumin denaturation technique which was studied according to the method of Leela Prakash and Mohan Dass, (2010). The reaction mixture consisted of test extracts and 1% aqueous solution of bovine albumin fraction, pH of the reaction mixture was adjusted using a small amount of 1N HCl. The sample extracts were incubated at 37 ºC for 20 min and then heated to 51°C for 20 min, after cooling the samples the turbidity was measured at 660nm. (UV Visible Spectrophotometer Model 371, Elico India Ltd). The experiment was performed in triplicate. The Percentage inhibition of protein denaturation was calculated as follows:

$$\text{Percentage inhibition} = \frac{(\text{Abs Control} - \text{Abs Sample}) \text{ X } 100}{\text{Abs Control}}$$

2.3 Statistical Analysis

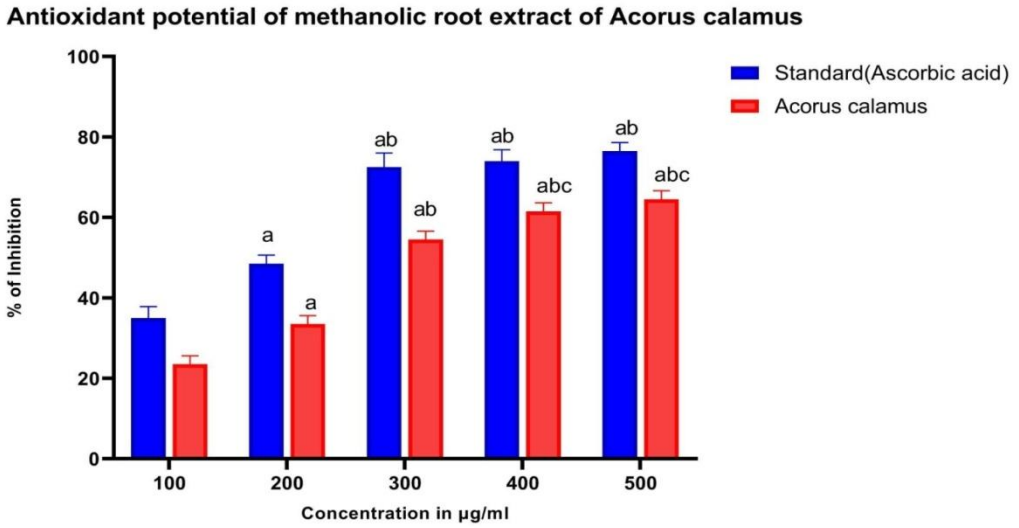
The triplicate analysis results of the experiments performed on methanolic root extract of *Acorus calamus* were expressed as mean ± standard deviation. Results were investigated statistically by a two-way analysis of variance (ANOVA) and significant differences between the mean values were measured using Tukey's multiple range test using Graph Pad Prism version 5. The results with the $p<0.05$ level were considered to be statistically significant.

3. RESULTS

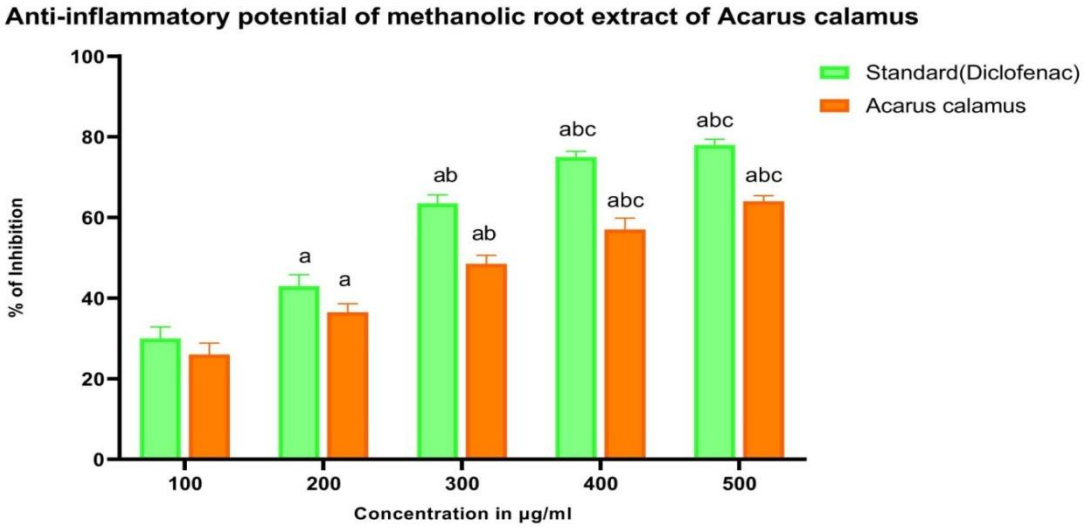
Table 1 represents the results of the phytochemical screening test done on the methanolic root extract of *Acorus calamus*. ++ denotes high presence, + denotes presence and - denotes absence of the phytochemicals in the extract. Amino acids and flavonoids have their high presence, proteins, terpenoids, steroids, saponins are present and Alkaloids and Carbohydrates are absent in the extract.

Table 1. Phytochemical screening test

Serial No	Phytochemicals	Presence
1	Amino Acid	++
2	Protein	+
3	Alkaloids	-
4	Terpenoids	+
5	Steroids	+
6	Carbohydrates	-
7	Saponins	+
8	Flavonoids	++



Graph 1. Represents antioxidant potential of methanolic root extract of *Acorus calamus* by DPPH Assay against the standard Ascorbic acid. X axis represents the concentration in µg/ml and Y axis represents the inhibitory potential of the extracts.Red bar denotes Methanolic root extract of *Acorus calamus* and blue bar denotes standard drug(Vitamin C).Each bar represents Mean ± SEM of 3 independent observations.Significance at p <0.05



Graph 2. Represents antiinflammatory potential of methanolic root extract of *Acorus calamus* by Albumin Denaturation Assay. X axis represents the concentration in µg/ml and Y axis represents the inhibitory potential of the extracts. Orange denotes Methanolic root extract of *Acorus calamus* and green denotes standard drug which is Diclofenac..Each bar represents Mean ± SEM of 3 independent observations.Significance at $p < 0.05$

Phytochemical screening tests of the methanolic root extract of *Acorus calamus* shows that there is a high presence of amino acids, flavonoids, proteins, terpenoids, steroids and saponins. It was observed that *Acorus calamus* has both antioxidant (IC_{50} of = 295 µg/ml) while comparing with the standard drug Vitamin C and as well as anti-inflammatory potentials (IC_{50} =310 µg/ml) as compared to the standard drug Diclofenac and the activity is compared with their respective standards shows that there is an increased rate of inhibition in a dose dependent manner.

4. DISCUSSION

Methanolic root extract of *Acorus calamus* was found to be rich in Alkaloids, Flavonoids ,Terpenoids, Sapanoids, Steroids and Phlobatannin.(Table 1) The extract also possesses significant antioxidant potential(Ic50 of *Acorus calamus* = 295 µg/ml) as compared with the standard Vitamin C(Ic50 = 210 µg/ml).(Graph 1) The anti inflammatory potential of the extract(Ic 50=310 µg/ml) was analysed and compared with standard drug- Diclofenac.(Ic 50=240µg/ml)(Graph 2)

Phytochemicals are secondary metabolites which are present only among plants. They possess various biologically active compounds that protect and help in normal functioning of the human body [37]. The presence of phytochemicals like alkaloids, saponins, Flavonoids indicates that the extract is potential for further in vitro analysis like antioxidant and anti inflammatory potentials.

Antioxidant potential of the methanolic root extract of *Acorus calamus* was determined by DPPH Free radical scavenging assay. Free radicals are molecules possessing an unpaired electron emerging by oxidative stress [38]. Phenolic compounds have great importance in free radicals scavenging potential. Researchers found that the role of flavonoids, alkaloids and other phenolic compounds in scavenging the free radicals and the antioxidant potential [39].

Effect of antioxidants on DPPH activity was considered to be due to their hydrogen donating ability [40]. Results obtained in study show that methanolic root extract of *Acorus calamus* has significant antioxidant activity (as compared with Vitamin C). Further studies may be needed to find out potential health benefits of extract in prevention of scavenging of free radicals.

A dose dependent Albumin denaturation inhibitory potential was observed for extract and standard drug. In the present study, the standard drug, Diclofenac, showed greater percentage of inhibition than the extract at the same concentration. The results revealed that standard drugs are the most potential drugs, but the

potential of extract can be increased by further purification of bioactive constituents. Many research on other bioactivities of *Acorus calamus* done by researchers has reported a slightly more inhibition of hemolysis exhibited by the extract (76.%) than the standard drug(72.8%) [10].

The results revealed that standard drug Diclofenac is the most potent drug compared to extracts. In future, the bioactive molecule from the extract can be purified and the purified phytochemicals like alkaloids, saponins, flavonoid can be tested for antioxidant and anti-inflammatory potential. Further studies are needed to find the potential health benefits of the extract. Instead of root extract, other parts can be used to obtain different results and also can be done in large scale size.

5. CONCLUSION

The bioactive compounds found in medicinal plants remain as an important component of research for the development of new drugs with potential to fight against several diseases and disorders. *Acorus calamus* Linn has been traditionally used as a Folk medicine. Anti inflammatory and antioxidant potential of the extract has been proved from the study. Further research needs to be done to analyse toxicity and to include it in drug formation for the betterment of mankind.

SOURCE OF FUNDING

The present study was supported by the following agencies

- Saveetha Dental College,
- Saveetha Institute of Medical and Technical Science,
- Saveetha University
- Durai Dental Clinic, Dharmapuri.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

ACKNOWLEDGEMENT

The authors would like to thank the Department of Biochemistry, Saveetha Dental College and Hospitals, Saveetha Institute of medical and technical Sciences, Saveetha University for providing research laboratory facilities to carry out the work.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Akhtar N, Ihsan-ul-Haq, Mirza B. Phytochemical analysis and comprehensive evaluation of antimicrobial and antioxidant properties of 61 medicinal plant species [Internet]. Arabian Journal of Chemistry. 2018;11:1223–35.
   Available:http://dx.doi.org/10.1016/j.arabjc.2015.01.013
2. Rajput SB, Tonge MB, Mohan Karuppayil S. An overview on traditional uses and pharmacological profile of *Acorus calamus* Linn. (Sweet flag) and other Acorus species [Internet]. Phytomedicine. 2014; 21:268–76.
   Available:http://dx.doi.org/10.1016/j.phymed.2013.09.020
3. Saxena M, Shakya AK, Sharma N, Shrivastava S, Shukla S. Therapeutic Efficacy of Rosa damascena Mill. on Acetaminophen-Induced Oxidative Stress in Albino Rats [Internet]. Journal of Environmental Pathology, Toxicology and Oncology. 2012;31:193–201.
   Available:http://dx.doi.org/10.1615/jenvironpatholtoxicoloncol.v31.i3.10
4. Muthuraman A, Singh N. Acute and sub-acute oral toxicity profile of *Acorus calamus* (Sweet flag) in rodents [Internet]. Asian Pacific Journal of Tropical Biomedicine. 2012;2:S1017–23.
   Available: http://dx.doi.org/10.1016/s2221-1691(12)60354-2
5. Gilani AUH, Shah AJ, Ahmad M, Shaheen F. Antispasmodic effect of *Acorus calamus* Linn. is mediated through calcium channel blockade. Phytother Res. 2006;20(12): 1080–4.
6. Kamil SS, Hameed I, Hamza LF. Acoruscalamus: Parts used, Insecticidal, Anti-Fungal, Antitumour and Anti-Inflammatory Activity: A Review [Internet]. International Journal of Pharmaceutical Quality Assurance. 2017;8.

Available:http://dx.doi.org/10.25258/ijpqa.v8i03.9578

7. Fujita S-I, Enomoto Y, Suemitsu R, Fujita Y. Miscellaneous Contributions to the Essential Oils of the Plants from Various Territories. XXVIII. : On the Components of the Essential Oils of *Acorus Calamus* LINN. var. angustatus BESS [Internet]. Yakugaku Zasshi. 1971;91:571–4. Available:http://dx.doi.org/10.1248/yakushi1947.91.5_571
8. Dong W, Yang D, Lu R. Chemical constituents from the rhizome of *Acorus calamus* L. Planta Med. 2010;76(5):454–7.
9. Gunavathy NG, Megala NN. screening Of Phytochemical Constituents in Solvent Extracts of *Acorus Calamus* [Internet]. Kongunadu Research Journal. 2014;1:38–41. Available: http://dx.doi.org/10.26524/krj9
10. Verma A, Department of chemistry, Govt. Science & Commerce college, Bhopal B, (India) 462003. Phytochemical Examination and in Vitro Free Radicl Scavinging Activity of Rhizome of *Acorus Calamus* Linn. Family Araceae [Internet]. International Journal of Scientific Research. 2012;3:54–5. Available:http://dx.doi.org/10.15373/22778179/jan2014/18
11. Awuchi CG. Medicinal Plants, Bioactive Compounds, and Dietary Therapies for Treating Type 1 and Type 2 Diabetes Mellitus [Internet]. Pharmacognosy - Medicinal Plants [Working Title]; 2021. Available:http://dx.doi.org/10.5772/intechopen.96470
12. Liu X, Zhou L, Liu Z, Du S. Identification of Insecticidal Constituents of the Essential Oil of *Acorus calamus* Rhizomes against Liposcelis bostrychophila Badonnel [Internet]. Molecules. 2013;18:5684–96. Available:http://dx.doi.org/10.3390/molecules18055684
13. Kumar U, Mishra M, Prakash V. Assessment of antioxidant enzymes and free radical scavenging activity of selected medicinal plants [Internet]. Free Radicals and Antioxidants. 2012;2:58–63. Available:http://dx.doi.org/10.5530/ax.2012.3.8
14. Muchtaromah B, Ahmad M, SEK, AYM, A VL. Phytochemicals, Antioxidant and Antifungal Properties of *Acorus calamus*, Curcuma mangga, and Allium sativum [Internet]. KnE Life Sciences. 2017; 3:93. Available:http://dx.doi.org/10.18502/kls.v3i6.1119
15. Parab RS, Mengi SA. Hypolipidemic activity of *Acorus calamus* L. in rats. Fitoterapia. 2002;73(6):451–5.
16. Choi H-J, Youngsan University. Anti-Inflammatory Effect Of Nandina Domestica Thunb Extracts Possessing Anti-Oxidant Property By Regulating MAPKs Signaling In LPS-Induced Inflammatory Cells [Internet]. Journal of Alternative, Complementary & Integrative Medicine. 2020;6:1–7. Available: http://dx.doi.org/10.24966/acim-7562/100114
17. Wu F, Zhu J, Li G, Wang J, Veeraraghavan VP, Krishna Mohan S, et al. Biologically synthesized green gold nanoparticles from Siberian ginseng induce growth-inhibitory effect on melanoma cells (B16). Artif Cells Nanomed Biotechnol. 2019;47(1):3297–305.
18. Chen F, Tang Y, Sun Y, Veeraraghavan VP, Mohan SK, Cui C. 6-shogaol, a active constiuents of ginger prevents UVB radiation mediated inflammation and oxidative stress through modulating NrF2 signaling in human epidermal keratinocytes (HaCaT cells). J Photochem Photobiol B. 2019;197:111518.
19. Li Z, Veeraraghavan VP, Mohan SK, Bolla SR, Lakshmanan H, Kumaran S, et al. Apoptotic induction and anti-metastatic activity of eugenol encapsulated chitosan nanopolymer on rat glioma C6 cells via alleviating the MMP signaling pathway [Internet]. Journal of Photochemistry and Photobiology B: Biology. 2020;203:111773. Available:http://dx.doi.org/10.1016/j.jphotobiol.2019.111773
20. Babu S, Jayaraman S. An update on β-sitosterol: A potential herbal nutraceutical for diabetic management. Biomed Pharmacother. 2020;131:110702.
21. Malaikolundhan H, Mookkan G, Krishnamoorthi G, Matheswaran N, Alsawalha M, Veeraraghavan VP, et al. Anticarcinogenic effect of gold nanoparticles synthesized from Albizia lebbeck on HCT-116 colon cancer cell lines. Artif Cells Nanomed Biotechnol. 2020;48(1):1206–13.
22. Han X, Jiang X, Guo L, Wang Y, Veeraraghavan VP, Krishna Mohan S, et al. Anticarcinogenic potential of gold nanoparticles synthesized from

Trichosanthes kirilowii in colon cancer cells through the induction of apoptotic pathway. Artif Cells Nanomed Biotechnol. 2019; 47(1):3577–84.

23. Gothai S, Muniandy K, Gnanaraj C, Ibrahim IAA, Shahzad N, Al-Ghamdi SS, et al. Pharmacological insights into antioxidants against colorectal cancer: A detailed review of the possible mechanisms. Biomed Pharmacother. 2018; 107:1514–22.
24. Veeraraghavan VP, Hussain S, Balakrishna JP, Dhawale L, Kullappan M, Ambrose JM, et al. A Comprehensive and Critical Review on Ethnopharmacological Importance of Desert Truffles: Terfezia claveryi, Terfezia boudieri, and Tirmania nivea [Internet]. Food Reviews International. 2021;1–20. Available:http://dx.doi.org/10.1080/87559129.2021.1889581
25. Sathya S, Ragul V, Veeraraghavan VP, Singh L, Niyas Ahamed MI. An in vitro study on hexavalent chromium [Cr(VI)] remediation using iron oxide nanoparticles based beads. Environmental Nanotechnology, Monitoring & Management. 2020;14:100333.
26. Yang Z, Pu M, Dong X, Ji F, Priya Veeraraghavan V, Yang H. Piperine loaded zinc oxide nanocomposite inhibits the PI3K/AKT/mTOR signaling pathway via attenuating the development of gastric carcinoma: In vitroandin vivostudies. Arabian Journal of Chemistry. 2020;13 (5):5501–16.
27. Rajendran P, Alzahrani AM, Rengarajan T, Veeraraghavan VP, Krishna Mohan S. Consumption of reused vegetable oil intensifies BRCA1 mutations. Crit Rev Food Sci Nutr. 2020;1–8.
28. Barma MD, Muthupandiyan I, Samuel SR, Amaechi BT. Inhibition of Streptococcus mutans, antioxidant property and cytotoxicity of novel nano-zinc oxide varnish. Arch Oral Biol. 2021;126: 105132.
29. Samuel SR. Can 5-year-olds sensibly self-report the impact of developmental enamel defects on their quality of life? Int J Paediatr Dent. 2021;31(2):285–6.
30. Samuel SR, Kuduruthullah S, Khair AMB, Shayeb MA, Elkaseh A, Varma SR. Dental pain, parental SARS-CoV-2 fear and distress on quality of life of 2 to 6 year-old children during COVID-19. Int J Paediatr Dent. 2021;31(3):436–41.
31. Tang Y, Rajendran P, Veeraraghavan VP, Hussain S, Balakrishna JP, Chinnathambi A, et al. Osteogenic differentiation and mineralization potential of zinc oxide nanoparticles from Scutellaria baicalensis on human osteoblast-like MG-63 cells [Internet]. Materials Science and Engineering: C. 2021; 199:111656. Available:http://dx.doi.org/10.1016/j.msec.2020.111656
32. Yin Z, Yang Y, Guo T, Veeraraghavan VP, Wang X. Potential chemotherapeutic effect of betalain against human non-small cell lung cancer through PI3K/Akt/mTOR signaling pathway. Environ Toxicol. 2021; 36(6):1011–20.
33. Veeraraghavan VP, Periadurai ND, Karunakaran T, Hussain S, Surapaneni KM, Jiao X. Green synthesis of silver nanoparticles from aqueous extract of Scutellaria barbata and coating on the cotton fabric for antimicrobial applications and wound healing activity in fibroblast cells (L929). Saudi J Biol Sci. 2021; 28(7):3633–40.
34. Mickymaray S, Alfaiz FA, Paramasivam A, Veeraraghavan VP, Periadurai ND, Surapaneni KM, et al. Rhaponticin suppresses osteosarcoma through the inhibition of PI3K-Akt-mTOR pathway. Saudi J Biol Sci. 2021;28(7):3641–9.
35. Teja KV, Ramesh S. Is a filled lateral canal – A sign of superiority? [Internet]. Journal of Dental Sciences. 2020;15:562–3. Available:http://dx.doi.org/10.1016/j.jds.2020.02.009
36. Kadanakuppe S, Hiremath S. Social and Behavioural Factors Associated with Dental Caries Experience among Adolescent School Children in Bengaluru City, India [Internet]., British Journal of Medicine and Medical Research. 2016;14:1–10. Available:http://dx.doi.org/10.9734/bjmmr/2016/24021
37. Bolanle JD, James, Bolanle D. Phytochemical Screening, and in-Vitro Antioxidant Activities in Different Solvent Extracts of Vitex Doniana Leaves, Stem Bark and Root Bark [Internet]. American Journal of Biomedical and Life Sciences. 2014;2:22. Available:http://dx.doi.org/10.11648/j.ajbls.20140201.14
38. Ahmad R. Free Radicals, Antioxidants and Diseases. BoD – Books on Demand. 2018; 148.

39. Chaubey P, Archana, Prakash O, Rai K, Kumar R, Pant AK. in vitro Antioxidant Activity and Total Phenolic Content of Rhizome Extracts from *Acorus calamus* Linn [Internet]. Asian Journal of Chemistry. 2017;29:2357–60. Available:http://dx.doi.org/10.14233/ajchem.2017.20657

40. Lee CY, Sharma A, Semenya J, Anamoah C, Chapman KN, Barone V. Computational Study of -Substituent Effects on Antioxidant Activities of Phenolic Dendritic Antioxidants. Antioxidants (Basel) [Internet]. 2020;9(3). Available:http://dx.doi.org/10.3390/antiox9030189

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/79887