Journal of Biochemistry and Biophysics
ISSN: 2576-7623
Volume 2 | Issue 1

Research Article Open Access

# Kaempferol 3-glucoside from *Azadirachta indica*

**Pasupuleti Sreenivasa Rao[*1,2], Gangapatnam Subramanayam[3], Perali Ramu Sridhar[4]**

[1]Research Scientist, Department of Advanced Research Centre, Narayana Medical College, Nellore, AP-524002, India
[2]Research Advisory Professor, Narayana College of Pharmacy, Nellore, AP-524002, India
[3]Director/cardiologist, Department of Cardiology/ Department of Advanced Research Centre, Narayana Medical College, Nellore, AP-524002, India
[4]Associate Professor, School of Chemistry, University of Hyderabad, Prof. C. R. Rao Road, Gachibowli Hyderabad, 500046, India

[*]**Corresponding author:** Sreenivasa Rao P, Research Scientist, Department of Advanced Research Centre, Narayana Medical College, Chinthareddypalem, Nellore, AP-524002, India, Fax: +91-0861-2317962, Tel: +91-0861-2317963, E-mail: sraopasupuleti@yahoo.com

**Citation:** Pasupuleti Sreenivasa Rao , Gangapatnam Subramanayam, Perali Ramu Sridhar (2018) Kaempferol 3-glucoside from *Azadirachta indica*. J Biochem Biophy 2(1): 102

**Received Date:** July 20, 2018 **Accepted Date:** November 27, 2018 **Published Date:** November 29, 2018

## Abstract

World population up to 80 percent relies on traditional medicine. Currently use of medicinal plant/herbal medicine for treating various disorders and diseases is quickly evolving. Due to their less side effects. *Azadirachta indica* (Neem) is one such important medicinal plant widely used in ethno traditional medicine for centuries, which is cosmopolitan in distribution and belongs to the family *Meliaceae*. In India, it is extensively used in several native medical practices. Its vegetative parts comprising of leaves, flowers, fruits, seeds and bark known to possess numerous bioactive molecules, so fondly called as store house of phytochemicals. Its chemical composition is complex and many studies reveal effective characterization and separation of bioactive compounds with significant biological activities. However, in the current study, we identified, the presence of flavonol glucoside in the various vegetative parts of neem extract using LC–MS (Liquid Chromatography and Mass Spectroscopy) spectral analysis. Moreover for the first time, we report the presence of major flavonol glucosides like kaempferol-3-glucoside ($C_{21}H_0O_{11}$) from the vegetative parts of A. *indica* collected from Tirumala Hills, Eastern Ghats.

**Keywords:** Flavonoids; Kaempferol-3-Glucoside; *Azadirachta indica*; Natural Products; LC –MS

## Introduction

Studies reveal that world population up to 80 percent relies on traditional medicine. Predominantly, the usage of medicinal plant/herbal medicine for treating various disorders and diseases is rapidly evolving and presumed with less side effects [1]. The active components existing in these Medicinal /herbal plants have been shown to efficiently hinder the disease or disorder symptoms in a synergistic manner. This active components from these Medicinal /herbal plants may comprise of polysaccharides, pigments, steroids, terpenoids, flavonoids and alkaloids etc. Earlier studies demonstrated that Medicinal/herbal plant extracts and purified molecules have significant effects in controlling and eradicating various diseases and disorders [1-8].

*A. indica* is referred as "village pharmacy" and widely employed in various health practices in rural India. It is an important medicinal plant, belongs to the family *Meliaceae*. It is distributed in cosmopolitan, indigenously covering tropics, starting from Asia to Africa [9-12]. It has numerous names, commonly called as 'Neem tree', or nature's 'drug store' or 'store house of phytochemicals'. Due to its wide spread and importance in ayurvedic medicine A. indica has been a potential target for extensive phytochemical investigations [10-11]. A. indica has been explored for centuries, employed in several native ethno-traditional medicinal health practices, and also its vegetative parts like, roots, leaves, bark, seeds and flowers have been used to treat various acute and chronic diseases and disorders [9-12]. These vegetative parts contain diverse range of phytochemicals with potential biological and pharmacological activities. Some of the purified bio-active compounds from *A. indica* are reported to exhibit anti-cancer, antimalarial, anti-bacterial, anti-fungal, anti-viral and anti-inflammatory properties, while few reported as insecticidal; larvicidal and spermicidal as well [9-12].

To date nearly 300 plus bioactive molecules have been reported from *A. indica*, which are chemically diverse, with their complex structures [9,12,13]. These are divided into isoprenoids, and non-isoprenoids. The isoprenoids are composed of diterpenoids,

triterpenoids, vilasinins, limonoids, and C-secomeliacins. The nonisoprenoids are composed of proteins, polysaccharides, sulphur compounds, polyphenolics, dihydrochalcone, coumarin, tannins and aliphatic compounds [9, 12, 14]. Thus, from the above reports it is clearly understood that *A. indica* has been effectively studied, characterized, and structurally elucidated in most African and Asian species [9-12]. However, there were no reports on the chemical composition of South Indian species especially from the region of Eastern Ghats. Hence the present study is undertaken to assess presence of the bioactive molecules like kaempferol-3-glucoside from *A. indica*.

## Materials and Methods

### Plant Collection

The germplam of *A.indica* was collected during the month of March, 2017 from the Tirumala hills, Eastern Ghats (Andhra Pradesh) of India, which was authenticated by native taxonomist. The collected germplasm (leaves, bark and roots) were subjected to shade dry as per described protocols [3-5]. After complete drying the germplasm was crushed and grinded in a mixer thoroughly until a fine powder is obtained.

### Preparation of A. indica Extracts

Initially aqueous extracts were prepared with the collected germplasm that comprised of leaves, bark and roots using Soxhlet apparatus. Approximately 15 gram of the powder of various vegetative parts of *A.indica* was taken separately and packed into sterile cloth, and placed in a thimble. The round bottom flask was filled with double distilled water (200 mL), and attached to a Soxhlet extractor and condenser on a heating mantle. The side arm was insulated with glass wool. With the help of heating mantle, the water was heated to above its boiling point (120°C) making water to pass through the apparatus to the condenser, where condensation takes place and trickles into the reservoir containing the thimble. Once the water reaches the limiting point in the siphon, it further pours back into the flask, and the fresh cycle begins again. The process was performed for 72 hours. After completion, the obtained water extract was further subjected to evaporation in a distillation unit, to obtain a minimal yield of extracted plant material up to 7 to 9 mL in the falcon tubes. Later these falcon tubes were placed in boiling water bath at 60˚C to eliminate the moisture content. The extract was filtered and concentrated, and the residue was dissolved in sterile water and filtered and was kept refrigerated until use. The concentration of the extract was calculated based on the dry weight per unit volume according to the described procedures.

### LC-Mass Spectral Analysis

The neem-extracts were subjected to chemical fingerprinting using LC-Mass spectral analysis as previously described [3]. The LC-MS (Liquid Chromatography and Mass Spectroscopy) was performed in SHIMADZU-LC- MS (Model: 2010A), using the solvents like methanol and water in combination, a gradient procedure was followed, using RP-C18 analytical column [240 mm× 2 cm] with a flow rate of 0.5 ml/min respectively. The extract samples were nebulized with nitrogen gas and the ion mass (Electro Spray Ionization) of the peaks were recorded in both positive mode and negative mode [3].

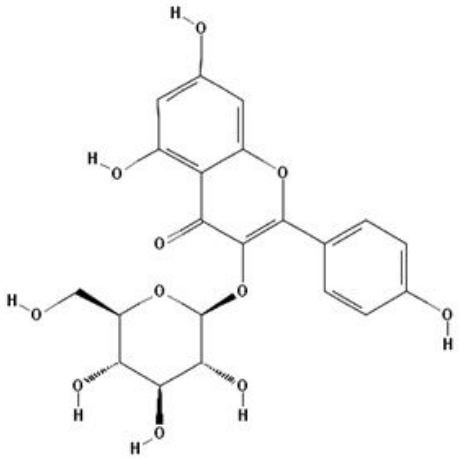
## Results

Flavonoids are prominent plant secondary metabolites found in most plants. They represent with a 15-carbon molecular framework, consisting of two phenyl rings (A & B) and one heterocyclic ring (C), and commonly abbreviated as C6-C3-C6, according to the IUPAC nomenclature. Very often the flavonoid units are attached to glycosides which are generally resembling glucose. These glucosides are commonly present in most plants. Glucose is generated when glucoside is hydrolysed by means of certain chemical degeneration, or fermentation process or enzyme process. Flavonoids in combination with glucosides results in the formation of flavonol glucosides. In other words they are also termed as phenolic compounds that contain a flavonoid moiety which is *O*-glycosidically connected to carbohydrate moiety at the C3-position. Moreover, if the glycone group of a glycoside is glucose, then it is termed as glucoside.

In the current study we assessed the distribution pattern of flavonol glucosides like Kaempferol 3-galactoside in the vegetative parts of *A. indica*. The Plant germplasm were collected from Eastern Ghats (Andhra Pradesh, India), shade dried, subjected to grinding and made fine powder. Later powdered material from various vegetative parts were extracted with water (aqueous) using soxhlet apparatus and water extracts were obtained. Further, these water extracts were sterile filtrated individually and subjected to LC-MS spectral analysis. The LC-MS (ESI) was employed to acquire chemical finger print profiles of aqueous extracts of *A. indica*. The LC-MS spectral profile data reported the presence of kaempferol-3-glucoside in the extracts, exhibiting with the protonated molecular ion peaks, at respective m/z observed in both positive mode and as well as in the negative mode (Figure 2A, B, 4A and B) (Table 1). The figure demonstrates the structure and molecular formula of kaempferol-3-glucoside ($C_{21}H_{20}O_{11}$) (Figure 1A).

| S. No | Name of the Identified Molecule in the LCMS spectra | Molecular formula | Mass (*m/z*) | Presence/Absence of molecule in the LC MS spectra of Root extract | | Presence/Absence of molecule in the LC MS spectra of Bark extract | | Presence/Absence of molecule in the LC MS spectra of Leaf extract | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | positive mode | negative mode | positive mode | negative mode | positive mode | negative mode |
| 1 | Kaempferol 3-glucoside | $C_{21}H_{20}O_{11}$ | 448.3 | yes | yes | yes | no | yes | yes |

**Table 1.** The distribution pattern of Kaempferol 3-glucoside from various vegetative parts of *A. indica*



**Kaempferol 3-glucoside $C_{21}H_{20}O_{11}$ (Mass 448.38 m/z)**

**Fig 1 .** The structural presentation of identified flavonol glucoside from *A. indica*

## Root Extract

The LC-MS spectral data of crude Aqueous root extract of *A. indica* displays the presence of a molecular ion peak of kaempferol-3-glucoside ($C_{21}H_{20}O_{11}$) at 448.3 m/z. The proton¬ated molecular ion peaks of kaempferol-3-glucoside was recorded in positive mode and as well as in negative mode (Figure 2A and B).

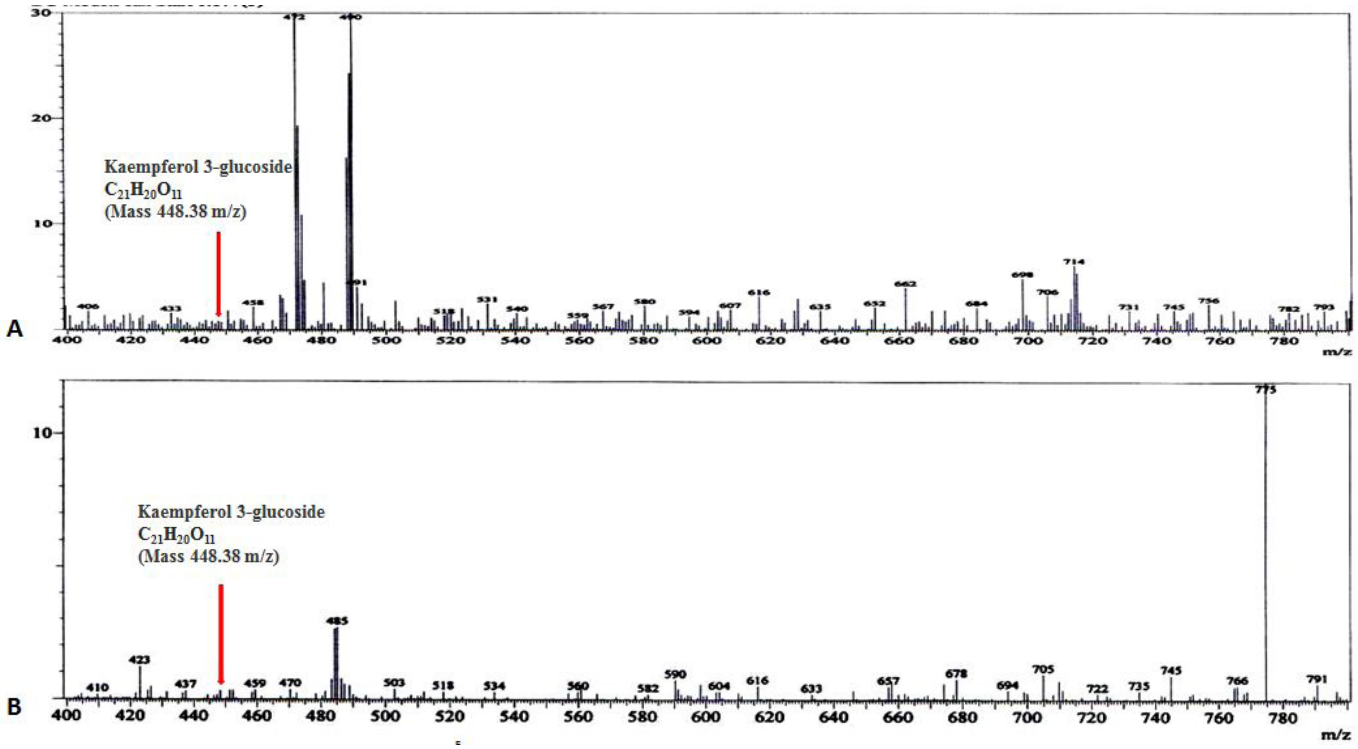



**Fig 2** A (Positive mode )-B (Negative mode ). The LC-MS spectral analysis (Positive mode and negative mode ) of Kaempferol 3-glucoside from the crude aqueous root extract of *A. indica*

## Bark Extract

Similar reports were noticed in bark extract too. The LC-MS spectral data of crude Aqueous bark extract of *A.indica* depicts the presence of a molecular ion peaks of kaempferol-3-glucoside ($C_{21}H_{20}O_{11}$) at 448.3 m/z. Interestingly, the protonated molecular ion peaks of kaempferol-3-glucoside were clearly recorded in positive mode and found to be completely absent in negative mode (Figure 3A,3B).

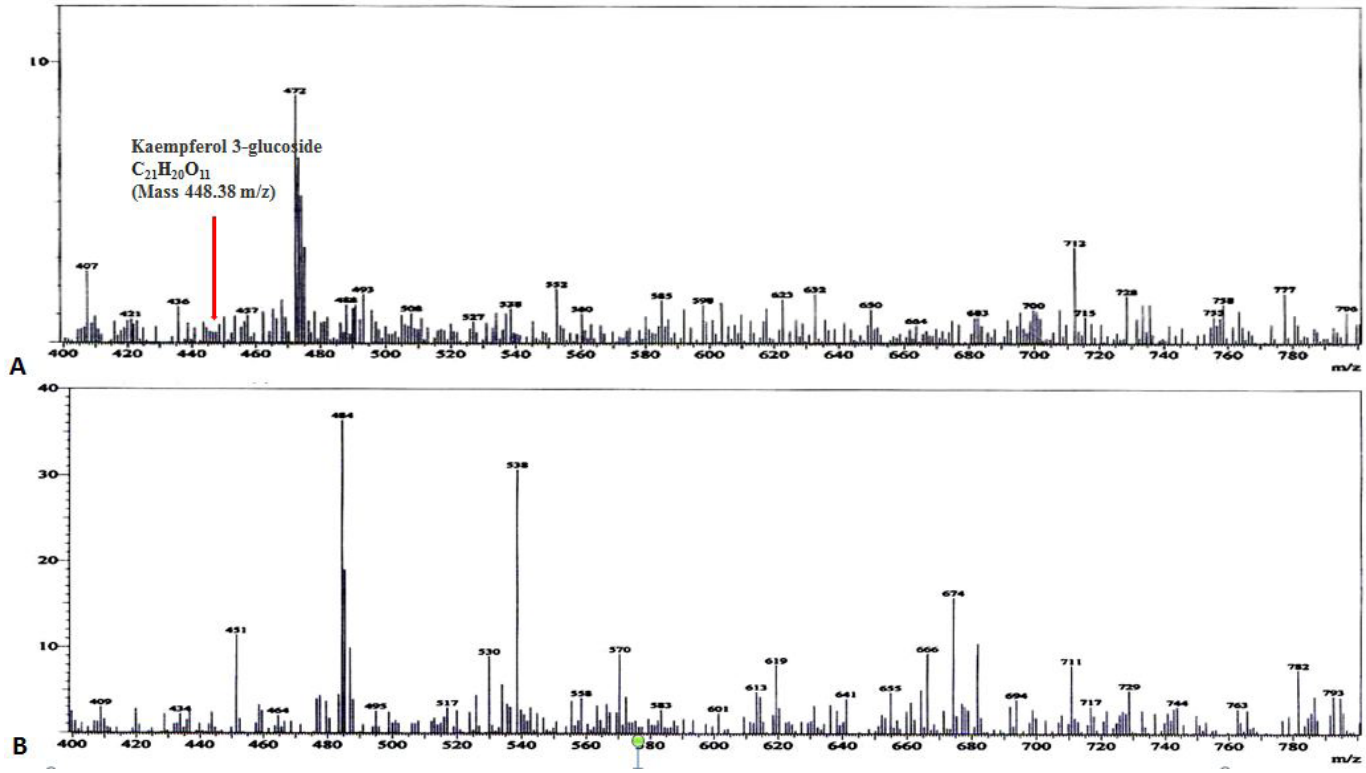



**Fig 3** A (Positive mode )-B (Negative mode ). The LC-MS spectral analysis (Positive mode and negative mode ) of Kaempferol 3-glucoside from the crude aqueous bark extract of *A. indica*

## Leaf Extract

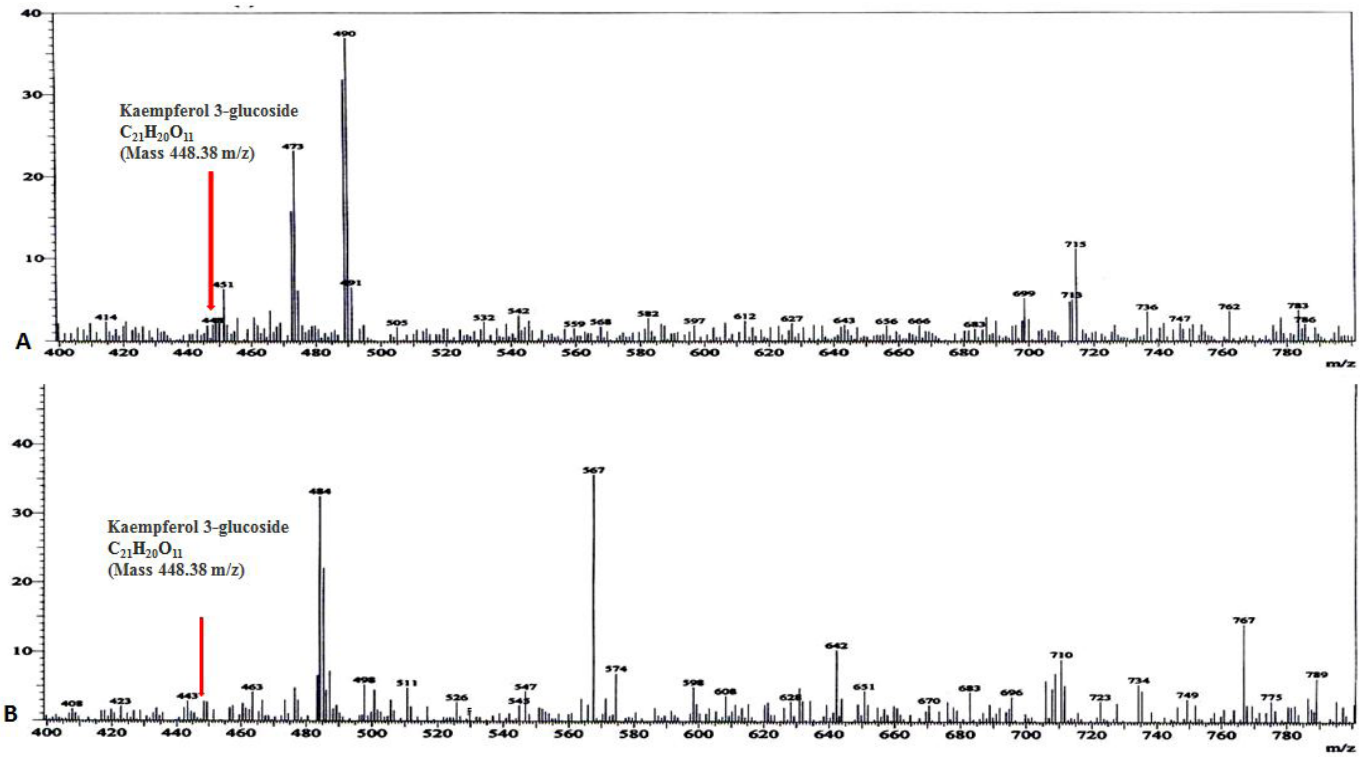



**Fig 4** A (Positive mode )-B (Negative mode ). The LC-MS spectral analysis (Positive mode and negative mode ) Kaempferol 3-glucoside from the of crude aqueous leaf extract of *A. indica*

The leaf extract of *A. indica* was also presented with similar findings. The LC-MS spectral data of crude Aqueous leaf extract of *A. indica* reports the presence of a molecular ion peaks of kaempferol-3-glucoside ($C_{21}H_{20}O_{11}$) at 448.3m/z. The proton¬ated molecular ion peaks of kaempferol-3-glucoside were clearly noticed in positive mode and as well as negative mode (Figure 4A and B).

## Discussion

The chromatographic techniques are widely employed in studying the natural or synthetic molecules that fight with various diseases and disorders. Recent advances in modern molecular biological tools like DNA sequencing, genetic engineering, gene targeting and transgenic methodologies demonstrated a new path to better understand and analyze the infections, diseases and disorders, which provide new options for developing new age therapeutics [15-18]. Currently, to combat diseases like cancer, and disorders like diabetes, several efficient drug development technologies are developed, through programs like in silico drug designing and synthesis of novel molecules [15-24]. However the problems still persist. Hence the discovery of alternative medicines are necessary.

Medicinal plants look as better option. As in ethno-traditional medicine, medicinal plants have been widely used to treat a variety of diseases and disorders [6-8]. Presently, usage of the medicinal plant/herbal extracts/formulations is quickly progressing, and assumed to have less side effects. One valid reason may be that the active ingredients present in this may be responsible for this effect. The active ingredients could be polysaccharides, pigments, steroids, terpenoids, flavonoids and alkaloids [1,5,6]. Moreover, screening these secondary metabolites has become an active field of study in the present circumstances, since they are potential sources for unique drugs [1,3]. Primarily these plant metabolites will be separated by various chromatographic techniques, by employing appropriate procedures, such as extraction, separation, purification, structural elucidation and quantification1-5. Initially various vegetative parts of the plant germplasm were collected, shade dried, lyophilized, further extracted with appropriate solvents by employing soxhlet extractor to separate bioactive compounds. After extraction, desirable bioactive compounds were separated, purified, structure elucidated and quantified by employing appropriate chromatographic techniques. Recent studies states that there is an urgency to adopt and implement modern analytical tools for investigating bioactive compounds. Moreover usage of new chemical fingerprinting methods with analytical tools like LC-MS, which can produce quality results in short time. So modern chromatographic fingerprinting methods could be employed in identifying and validating various molecules that completely represent a particular plant or herb.

As mentioned earlier, in native ethno-traditional medicine *A. indica* is widely in various health practices. Its ingredients were well studied and characterized [11]. Patela et al 2016, classified neem components into two categories, namely Isoprenoids and non-isoprenoids. In the isoprenoids categeory the diterpenoids, triterpenoids and steroids were placed. The Falavanoids, coumarins, carbohydrates, proteins, hydrocarbons, fatty acids and esters, and other acids were placed under the category of non-isoprenoids. Further, the triterpenoids are divided into many types based on the removal of carbon atom either from the side chain or from the ring skeletal structure of the parent compound. The triterpenoids are further classified as protolimonoids, mononortriterpenoids, dinortriterpenoids, trinortriterpenoids, tetranortriterpenoids, pentanortriterpenoids, hexanortriterpenoids, octanortriterpenoids and nonanortriterpenoids. Furthermore tetranortriterpenoids were divided into two groups, namely ring-intact- tetranortriterpenoids and ring-seco-tetranortriterpenoids. The diterpenoids were further divided into two groups, such as podacarpanoids (margolone) and abeitanoids (sugiol). But the chemical composition in plants differs due to their geographical distribution, seasonal variations and other environmental factors [3]. In spite of its therapeutic importance, the chemical composition of *A.indica* species, distributed in Eastern Ghats has not been studied in detail. Therefore, the present investigation is carried out with an aim to report the favonol glucosides like kaempferol-3-glucoside ($C_{21}H_{20}O_{11}$) in *A. indica*. Thus, in the present study *A. indica* germplasm comprising of leaves, bark and roots has been collected, shade dried, subjected to grinding and made fine powder. Next, these powders were extracted with water in soxhlet apparatus and water extracts were obtained. Later the water extracts were sterile filtrated and sent to LC-MS spectral analysis. The respective molecular mass of the kaempferol-3-glucoside ($C_{21}H_{20}O_{11}$) have been recorded from various extracts with their positive and negative ion electrospray mass spectra (ESI-MS),and well as with their corresponding protonated and deprotonated pseudo-molecular ion peaks (Figure 2-4, Table 1).

Flavonoids, generally referred as di-phenyl propanes or C6-C3-C6 ring structures, which are found in large numbers in most plants and *A.indica* is one among them. Flavonoids are major classes of bioactive compounds, with wide range of biological and physiological activities [25,26]. Typically, flavonoids are attached to a variety of hydroxyl groups. In majority of the cases flavonoids will appear in conjugated forms or their hydroxyl groups associated with one or more sugar residues. Frequently they will be associated with carboxylic acids, amines, lipids and also connected with phenols. Studies reveal that flavonoids exhibit antioxidant activity, which is based on their linked structure, and also act as reducing agents, hydrogen-donating antioxidants and quenchers of singlet oxygen [26-28]. kaempferol 3-glucoside is an important flavonoid which has clinical importance. It is also known as astragalin and have been reported in several medicinal plants like *Cuscuta chinensis, Camellia sinensis, Cassia alata,* and *Centella asiatica* etc [29]. It has wide range of pharmacological activities like anti-inflammatory, antioxidant, neuroprotective, cardioprotective, anti-obesity, anti-osteoporotic, anti-cancer, anti-ulcer, and anti-diabetic properties. It regulates various pathways, by targeting various transcription factors like NF-κB, TNF-α, and TGF-β1; enzymes like iNOS, COX-2, PGE2, MMP-1, MMP-3,

MIP-1α, COX-2, PGE-2, HK2, AChe, SOD, DRP-1, DDH, PLCc1, and GPX; kinases like JNK, MAPK, Akt, ERK, SAPK, IκBα, PI3K, and PKCβ2; cell adhesion proteins like E-cadherin, vimentin PAR-2, and NCam. It also plays a key role in regulating various apoptotic and anti-apoptotic proteins like Beclin-1, Bcl-2, Bax, Bcl-xL, cytochrome c, LC3A/B, caspase-3, caspase-9, procaspase-3,procaspase-8, and IgE. It also regulates various inflammatory cytokines like SOCS-3, SOCS-5, IL-1β, IL-4, IL-6, IL-8, IL-13, MCP-1, CXCL-1, CXCL-2, and IFN-c [29]. However, there were no reports its presence in South Indian species of *A. Indica*, especially from the region of Eastern Ghats. Thus, in the current study various vegetative parts of *A. indica* were assessed to identify the favonol glucosides like kaempferol-3-glucoside ($C_{21}H_{20}O_{11}$), using new age chemical fingerprinting tools like LC-MS.

The structural representation of kaempferol-3-glucoside ($C_{21}H_{20}O_{11}$) has been displayed in Figure 1A along with the molecular formula. The LC-MS spectral data of various vegetative parts of crude aqueous extracts of *A.indica* clearly demonstrated the presence of flavonol glucosides like kaempferol-3-glucoside ($C_{21}H_{20}O_{11}$), in both positive mode and negative mode (Figure 2-4). Kaempferol-3-glucoside ($C_{21}H_{20}O_{11}$) was identified in all the extracts assessed (Table 1). The data revealed the presence of kaempferol-3-glucoside ($C_{21}H_{20}O_{11}$) in the spectral data and the molecular ion peaks was observed at 448.3 m/z respectively. The identified kaempferol-3-glucoside ($C_{21}H_{20}O_{11}$) was observed in either positive mode or negative mode or complete absent of in vegetative aqueous extracts. In root extracts of *A. indica* the proton¬ated molecular ion peaks of kaempferol-3-glucoside ($C_{21}H_{20}O_{11}$) was observed in both positive mode (Figure 2A) and as well as in negative mode (Figure 2B). Interestingly, incase of bark extract the protonated molecular ion peaks of kaempferol-3-glucoside ($C_{21}H_{20}O_{11}$) was clearly noticed in positive mode and completely absent in negative mode (Figure 3A and B). The leaf extract spectral data showed the presence of kaempferol-3-glucoside ($C_{21}H_{20}O_{11}$) both in positive and negative mode (Figure 3A and B). Moreover, the flavonol glucosides like kaempferol-3-glucoside ($C_{21}H_{20}O_{11}$) reported in the present study were correlated with other studies that demonstrated the presence of these molecules [29-30].

## Conclusion

Thus from the above study based on the qualitative analysis we conclude that presence of flavonol glucosides like kaempferol-3-glucoside ($C_{21}H_{20}O_{11}$) has been reported from various vegetative parts of *A. indica* collected from Tirumala hills, Eastren Ghats. However further studies are required for in depth quantitative analysis.

## References

1. Rao PS, Subrahmanyam G (2017) Induction of diabetes by alloxan in male wistar albino rats –a simplified methodology. Euro J Biomed Pharmace sci 6: 68-75.

2. Rao PS, Subrahmanyam G, Bhaskar M (2017) U-HPLC (Ultra-High-pressure Liquid Chromatography) Separation of Indole Alkaloid Strychnine. World J of Pharmace Res 4: 1022-35.

3. Rao PS, Prasad MNV (2008) Extraction, Purification and Characterization of Indole Alkaloids from *Strychnos wallichiana L.* – an Endangered Medicinal Plant from India. Medici Aroma Plant Sci Biotech 2: 63-7.

4. Rao PS, Ramanadham M, Prasad MNV (2009) Anti-proliferative and cytotoxic effects of *Strychnos nux-vomica* root extract on human multiple myeloma cell line - RPMI 8226. Food Chem Toxic 47: 283-8.

5. Rao PS, Prasad MNV (2013) The *Strychnos nux-vomica* root extract induces apoptosis in the human multiple myeloma cell line-U266B1. Cell Biochem Biophy 66: 443-50.

6. Satyanand V, Reddy CB, RamaMohan P, Kumar MR, Narayanaswamy DL, et al. (2013) Effects of Garlic extract (*Allium sativum*) in combination with Amlodipine in mild to moderate essential hypertensive patients: An Open randomized parallel group study. J Pharmace Res Dev 2: 181-8.

7. Satyanand V, Krishnan V, Ramalingam K, Rao PS, Priyadarshini S (2013) Blockade of voltage dependent calcium channels lowers the high blood pressure through ginger. Int J Analy, Pharmace Biomed Sci 2: 64-6.

8. Satyanand V, Venkat Krishnan, Madhavi D, Revathi, Indira S, et al. (2013) The effect of peppermint juice for indigestion among old age people- A preliminary study. J Pharmace Res Dev 2: 238-43

9. Akhila A, Rani K (1999) Chemistry of the neem tree (*Azadirachta indica A. Juss.*) Fortschritte der Chemie organischer Naturstoffe / Progress in the chemistry of organic natural products. Progres dans la chimie des substances organiques naturelles 78: 47-149.

10. Patela SM, Venkata KCN, Bhattacharyya P, Sethi G, Bishayee A (2016) Potential of neem (*Azadirachta indica L*) for prevention and treatment of oncologic diseases. Semin Cancer Biol 40-1: 100-15.

11. Gupta SC, Prasad S, Tyagi AK, Ajaikumar B, Kunnumakkara, et al. (2017) Neem (*Azadirachta indica*): An indian traditional panacea with modern molecular basis. Phytomedicine 34: 14-20.

12. Biswas K, Chattopadhyay I, Banerjee RK, Bandyopadhyay U (2002) Biological activities and medicinal properties of neem (*Azadirachta indica*). Curr Sci 82: 1336-45.

13. Subapriya R, Nagini S (2005) Medicinal properties of neem leaves: a review. Curr Med Chem Anti-Canc Agents 5: 149-6.

14. Brahmachari G (2004) Neem–an omnipotent plant: a retrospection. Chembiochem: 5: 408-21.

15. Suresh G, Gopi Krishna S, Nayudu N, Sravanthi M, Rao PS, et al. (2014) Contribution of cyclin D1 (CCND1) and E-cadherin (CDH1) alterations to colorectal cancer susceptibility: a case–control study. Tumor Biol 35: 12059-67.

16. Suresh G, Sravanthi M, Bulle S, Dasi D, Prathap BN, et al. (2016) Manganese-superoxide dismutase (Mn-SOD) overexpression is a common event in colorectal cancers with mitochondrial microsatellite instability. Tumor Biol 37: 10357-64.

17. Suresh G, Bulle S, Sravanthi M, Krishna MT, Nagesh N, et al. (2016) Association of Mitochondrial Displacement Loop Polymorphisms with Risk of Colorectal Cancer in South Indian Population. Mitochondrial DNA 28: 632-7.

18. Singh S, Kotakonda A, Kapardar R, Kankipati H, Rao PS, et al. (2015) Response of bacterioplankton to iron fertilization of the Southern Ocean, Antarctica. Front Microbiol 6: 863.

19.Chetan R, Veeresalingam B, Kumar KM, V, Teja PD, Rao PS (2013) A study on the clinical manifestations and the incidence of benign and malignant tumors in a solitary thyroid nodule. Int J Res Medi Sci Nov;1(4):429-434

20.Reddy S A, Dasu K, Venkat Krishnan, Reddy MR, Jithendra K, Rao PS (2013). Prevalence of asymptomatic bacteriuria and its antibiotic sensitivity in type-2 diabetic women along the sea coast. Int J Res Medi Sci 1(4):487-495

21.Rao PS, Muvva C, Geethanjali K, Babu BS, Kalashikam R (2012) Molecular docking and virtual screening for novel protein tyrosine phosphatase 1B (PTP1B) inhibitors. Bioinformation 8(17): 834-837.

22. Bola BR, Rao PS, Satish S (2017) Ligand Docking Based Identification of Novel Drug Analog for an Effective Treatment against Filaria , Haya: Saudi J. Life Sci. 2, -9: 335-348.

23.Avinash A, Swarupa SS, Siva K, Sirisha D, Riyaz S, kumar ND, Sreenivasulu M, Rao PS (2015). Design and Evaluation of Famotidine Floating Tablets. Int J Innov Pharmace Res 6 (1):440-445

24. Hymavathi, R, Suresh, G, Sumanth, K.M, Swapna, V.K, Sravanthi, M, Rao, P.S, Manjula, B, Varadacharyulu, N (2017). Therapeutic effect of green tea extract on alcohol induced hepatic mitochondrial DNA damage in albino wistar rats. J Adv Res 8(3): 289-295.

25. Cuyckens F, Claeys M. (2004). Mass spectrometry in the structural analysis of flavonoids. J Mass Spectrom. 39:1–15.

26. Cook NC, Samman S (1996). Flavonoids—chemistry, metabolism, cardioprotective effects, and dietary sources. J Nutr Biochem 7:66–76.

27. Moure A, Cruz JM, Franco D, Domınguez JM, Sineiro J, Domınguez H, Nu~nez MJ, Parajo JC (2001). Natural antioxidants from residual sources. Food Chem. 72:145–171.

28. Bravo L. 1998. Polyphenols: Chemistry, dietary sources, metabolism, and nutritional significance. Nutr Rev 56:317–333.

29. Riaz A, Rasul A, Hussain G, Zahoor MK, Jabeen F, et al. (2018) Astragalin: A Bioactive Phytochemical with Potential Therapeutic Activities. Advances in Pharmacological Sciences 2018: 9794625.

30. Keskes H, Belhadj S, Jlail L, Feki AE, Damak M, et al. (2017) LC-MS–MS and GC-MS analyses of biologically active extracts and fractions from Tunisian *Juniperus phoenice* leaves. Pharm Biol 55: 88-95.

31. Francescato LN, Debenedetti SL, Schwanz TG, Bassani VL, Henriques AT (2013) Identification of phenolic compounds in *Equisetum giganteum* by LC–ESI-MS/MS and a new approach tototal flavonoid quantification. Talanta 105: 192-203.