International Research Journal of Biological Sciences _____ ISSN 2278-3202
Vol. **8(5),** 1-7, May **(2019)** *Int. Res. J. Biological Sci.*

Investigating anti-oxidant and anti-diabetic activity of leaf extracts of *Justicia adhatoda* and *Murraya koenigii*

Mishra S.[1], Khatri M.[2] and Mehra V.[2*]
Department of Biomedical Science, Shaheed Rajguru College of Applied Sciences for Women, University of Delhi, Delhi-110096, India
varsha.mehra@rajguru.du.ac.in

Available online at: www.isca.in, www.isca.me
Received 25th October 2018, revised 4th April 2019, accepted 3rd May 2019

Abstract

Justicia adhatoda and Murraya koenigii are well known indigenous medicinal plants which have always been used in various Ayurvedic medicines. Where J.adhatoda is known to be effective against bronchitis, asthma, blood disorders, cardiac problems, fever; M.koenigii is known to be useful as laxative, curing persistent boils, vitamin and calcium deficiency as well as osteoporosis. Apart from their uses in Ayurvedic and Unani system, these plants are given no importance in Allopathy. In Ayurvedic system, both plants are also known to be used in the treatment of Diabetes, whose existence can be dated back to Vedic period. The disease is the combination of multiple disorders commonly presenting with the hyperglycemia and glucose intolerance due to lack of insulin, defective insulin activity or both. Till now there is no complete cure of the disease as it is chronic and patient having the disease has to live on lifetime medication. Today, more and more researchers are focusing on plant based treatment as it is safe and devoid of any toxic side effects. In this study, Ethanol and DCM leaf extracts of J.adhatoda and M.koenigii were subjected to in-vitro analysis of Anti-oxidant and Anti-diabetic activity. Both in-vitro assays are the preliminary biochemical tests Both plant extracts were compared and evaluated to find the possible bioactive compound responsible for the above mentioned properties.

Keywords: Ayurveda, *Justicia adhatoda, Murraya koenigii,* Phytochemical tests, Anti-oxidant activity, Anti-diabetic activity.

Introduction

Diabetes is one of the non-infectious diseases which has increasingly been a cause of concern even with the advancement of human civilization and continues to pose a burden in our society. The disease is characterized by hyperglycemia and glycosuria which are in part due to lack of insulin, defective insulin activity or both. Although it can be managed by using commercially available drugs, the treatment is often lifelong and requires rigorous lifestyle management. If undiagnosed, the disease leads to severe late stage complications such as neuropathy, retinopathy and nephropathy[1]. As per WHO, the current burden of diabetes is around 422 million people globally and it is estimated to be the 7th leading cause of fatality by the year 2030. India among several other developing countries is perhaps one of the highest contributors to this number[2]. Mostly, drugs used to lower the blood glucose level or raise insulin sensitivity have mild side effects which can be handled easily. Some hypoglycemic drugs like Saxagliptin, Alogliptin, Avandia, Pioglitazone are counterproductive and worsen the disease progression[3]. Whatever be the mode of action of the drug, the underlying mechanism is to prevent disease progression either by increasing the insulin production or decreasing gluconeogenesis. This can be achieved by up regulating the transcription of certain genes like AP2 which would then lead to the down regulation of FFA (Free Fatty Acid), TNF-α and leptin levels. However, this requires regular administration of the aforementioned drugs which ultimately add to the risk factor for pathies associated with liver and kidney. Furthermore, this could result in the conditional Hyperinsulinemia which may lead to Non-Insulin Dependent Diabetes Mellitus (NIDDM), also known as Type-2 Diabetes. The treatment regimen for Type-2 Diabetes is more rigorous in that a number of drugs have to be administrated (combination therapy) eventually with deleterious side effects[4]. Diabetes being a non- curable disease which primarily attacks our immune system results in an immunocompromised condition thereby making the body more prone to a number of other diseases such as Poly Cystic Ovarian Disease (PCOD), Tuberculosis, Breast cancer, etc.[3] This calls for an urgent need of new drug regimen for diabetes which not only would narrow down the profile of side effects caused by the existing drugs regimens but will also be helpful in eradicating the chronic disease.

Justicia adhatoda is a medicinal plant found commonly in the Indian sub-continents. It is known to be effective against various infectious disease like bronchitis, asthma, blood disorders, cardiac problems, fever, etc. The plant has been extensively used in Ayurveda for treating various disease including Diabetes, but so far no evidence has been reported for its Anti-diabetic activity in the scientific literature[5]. While, *Murraya koenigii*, also known as Curry leaf/tree, is a very popular condiment used regularly in Indian Cuisine. Several studies

support the anti-diabetic and anti-oxidant potential of various extracts of *Murraya koenigii.* Its use can be used traced back to the Vedic period wherein its medicinal properties were exploited in the traditional system of medicine. But literature relevant to its use in allopathic system of medicine is sparse[6,7]. In this article, the anti-oxidant and anti-diabetic properties of both plant leaves are evaluated. *In-vitro* assays are performed on the two types of leaf extracts (Ethanol and Dichloromethane (DCM)) for their anti-oxidant and anti-diabetic activity. The phytochemical profiling is done to speculate the most potent of all the compounds responsible for the ensuing effects. Further identification of the lead compound(s) is currently underway.

Materials and methods

Sample and Material collection: *Justicia adhatoda* leaves/plant were acquired from Kamla Nehru Ridge, New Delhi, during the month of October. *Murraya koenigii* leaves were acquired from the garden of college campus, Delhi. Samplewas verified by NISCAIR (NISCAIR/RHMD/Consult/ 2015/2907/100). All the solvents and chemicals used in biochemical tests were bought from Merck Millipore. DPPH, Alpha amylase and Acarbose were bought from SRL chemicals, India.

Extract preparation: Leaves of both plants were shade dried and extracts were prepared in Ethanol (EtOH) and Dichloromethane (DCM). 200g of the powdered dried whole plant was mixed in 2 separate conical flasks (2L volume) containing 1L of each solvent. Each flask was covered with foil and incubated at 37°C on magnetic stirrer for 24 hours. After incubation, the mixture was filtered via Vacuum pump and concentrated by using Rotary evaporator. Same process was repeated for preparing leaf extracts. Total extract yield was noted for future calculation. All extracts were assessed to determine their properties by different assays. All tests were repeated five times and the results were represented in terms of Mean ± Standard Deviation (SD).

Phytochemical Analysis: Qualitative tests for alkaloids, phenols, flavonoids, saponins, steroids, tannins and terpenoids were performed according to the protocol with slight modifications[8-11]. Quantitative analysis was performed for alkaloids, flavonoids, steroids[12], phenols and tannins[13-14].

Anti-oxidant Activity: Anti-oxidant activity was assessed by Phosphomolybdenum reduction assay and Free Radical Scavenging Assay; Phosphomolybdenum reduction assay, also known as reducing power assay and DPPH radical scavenging assay, commonly known as Percentage Free radical scavenging assay by methods described in Alam et al[11], Liyana-Pathirana and Shahidi[15] and Govindappa et al[16].

Phosphomolybdenum Reduction Assay: 1ml of extracts with varied concentration (100µg/ml to 500µg/ml) was incubated with 1ml of reagent solution (0.6M Sulfuric acid, 28mM Sodium phosphate and 4mM Ammonium molybdate) at 95°C for 90 minutes. After the colour development, the absorbance was noted at 695nm. The test was repeated for a series of concentration of Ascorbic acid (100mmol/ml to 1000mmol/ml), which was used as Standard. Standard curve was plotted (X axis representing the Ascorbic acid concentration and Y axis representing Absorbance of Standard at 695nm.) and values for the samples were obtained by extrapolating the results in the standard graph. Value of sample was determined in terms of Ascorbic acid Equivalent/Gram of sample[17].

DPPH radical scavenging assay: In this method, 1,1-diphenyl-2-picrylhydrazyl (DPPH), a stable free radical, was incubated with the series of extracts (100µg/ml to 500µg/ml). 0.1mM of DPPH solution was prepared in 100% methanol, from which 1ml of DPPH solution was mixed with 1ml of Extract. After 30 minutes incubation in dark, the absorbance was noted at 517nm. Ascorbic acid was used as standard in this assay[18].

Alpha-Amylase Inhibition Assay: For Anti-diabetic activity, Alpha amylase Inhibition Assay, was done in which plant extracts were incubated first with the α-amylase enzyme for 10 minutes at 37ºC and then with starch solution (1% w/v) for 10 minutes. Maltose was used as standard and different concentration of maltose was made (to plot standard curve) and incubated with DNS reagent for 10minutes at 37ºC in water bath. The entire assay was performed in phosphate buffer (pH 6.9)[19,20]. Absorbance was taken of standards and extracts at 540nm after diluting the content with distilled water. Enzyme Activity(EA) was calculated by plotting the Absorbance value obtained of all samples and controls (positive control contained enzyme and starch solution while negative control contained only starch; both were mixed with DNS reagent) and results were represented in % inhibition of alpha amylase enzyme, which was calculated as per the given formula.

$$\% \text{ Alpha amylase Inhibition} = 100 - (EA_P - EA_T)/EA_P \times 100$$

Where: EA_P - Enzyme Activity of Positive control

EA_T - Enzyme Activity of Test sample.

Results and discussion

Phytochemical Analysis: Quantitative analysis of leaf extracts of *M.koenigii* and *J.adhatoda* was done for Phenol, Flavonoids, Alkaloids and Tannins. Where, Ethanolic extracts of both plants have all phytoconstituents in varying quantity, DCM extracts did not contain Alkaloids but other contents in varying amount.

Anti-oxidant Activity: Phosphomolybdenum Assay: Ethanolic extract of *M.koenigii* showed exceptionally high reducing power than its DCM extract as well as *J.adhatoda* extracts. While Ethanolic extract of *J.adhatoda* also showed good reducing power, but the power is almost half as compared to *M.koenigii* Ethanolic leaf extract. DCM extracts of both plants have comparable reducing power and lower than their Ethanolic component.

Table-1: Phytochemical analysis of leaf extracts of *M.koenigii* and *J.adhatoda.*

Phytochemicals	*M.koenigi*i Extracts		*J.adhatoda* Extracts	
	Ethanol	DCM	Ethanol	DCM
Total Phenol content(mg GAE/g)	82±2.13	57±1.36	36±0.65	4±0.35
Total Flavonoid content(mg QE/g)	47±0.16	2±0.33	20±0.14	23±0.14
Total Alkaloid content(mg/g)	54±3.9	-	201±0.31	-
Total Tannin content(mg CE/g)	80±3.17	75±3.02	5±0.02	9.5±1.36

Where: DCM: Dichloromethane; ND: Not Discovered; GAE: Gallic acid Equivalent; QE: Quercetin Equivalent; CE: Catechin Equivalent; Values expressed as Mean ± SD (Standard Deviation).

Table-2: Total Antioxidant capacity of *Murraya koenigii* and *Justicia adhatoda* leaf fractions expressed as ascorbic acid equivalents (mmol/g of extract) by phosphomolybdenum method (Reducing Power Assay).

Conc. of extract (µg/ml)	*M.koenigii*		*J.adhatoda*	
	Ethanol (mmol/g)	DCM (mmol/g)	Ethanol (mmol AE/g)	DCM (mmol AE/g)
100	552±6.23	85±5.2	260±2.63	25 ± 1.76
200	1004±5.26	215±15.2	450±1.03	134 ± 2.63
300	1157±3.41	301±7.12	578± 0.31	182 ± 3.96
400	1502±2.13	375±1.02	735±7.0	349 ± 4.92
500	1653±11.23	430±1.97	815±3.21	500 ± 7.88

Values expressed are mean ± SD; DCM – Dichloromethane.

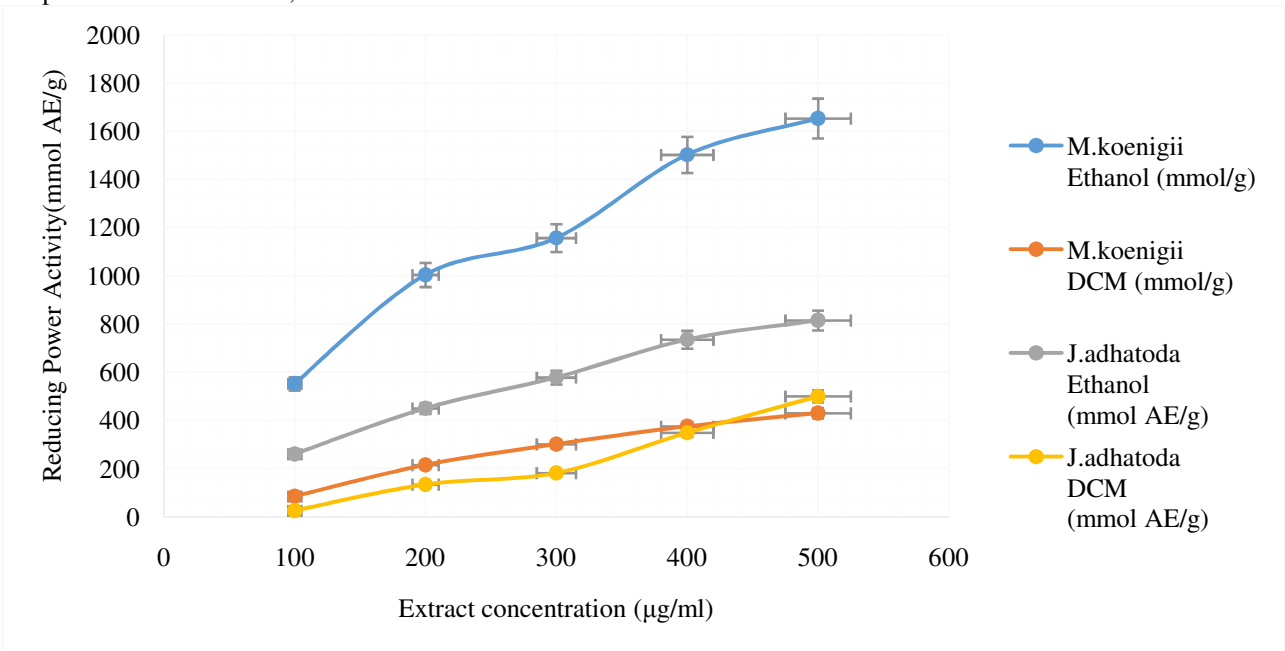

Figure-1: Graph depicting Reducing Power Activity of leaf extracts of *M.koenigii* and *J.adhatoda*. 1 unit of X axis represents 100µg/ml of extract concentration. 1unit of Y axis represents 200mmol of Ascorbic acid Equivalent/g of Reducing Power Activity.

DPPH Assay: Free radical scavenging activity of DCM extract of *M.koenigii* leaf came out to be best as compared to the other extracts. Surprisingly, *M.koenigii* Ethanolic extract was found out to be less active than Ethanolic extract of *J.adhatoda* leaf, which came out to be the second best extract. Activity of DCM extract of *J.adhatoda* was comparable to Ethanolic extract of *M.koenigii* leaf. IC_{50} of the % activity of all extracts have been calculated and shown in the last row of Table-3.

Table-3: Percentage Free Radical Scavenging activity by DPPH Assay of *M.koenigii* and *J.adhatoda* leaf extracts with Standard as Ascorbic acid.

Conc. of extract (µg/ml)	*M.koenigii*		*J.adhatoda*		Ascorbic acid (% activity)
	Ethanol (% activity)	DCM (% activity)	Ethanol (% activity)	DCM (% activity)	
100	15.2±0.32	21±5.32	26.83±1.56	15.47±0.21	9.79±0.13
200	22.5±1.3	37±7.37	29.72±0.95	20.18±3.66	19.59±0.344
300	27±3.4	53±3.78	38.27±0.76	26.49±1.14	29.38±4.08
400	39 ± 0.33	67±1.21	41.54±0.83	30.06±0.52	39.04±2.76
500	47±0.37	78±9.22	52.34±0.53	38.87±6.13	48.98±0.69
IC_{50}(µg/ml)	520±4.21	300±6.37	460±6.23	600±10.84	515±2.3

Values expressed are mean ± SD; DCM – Dichloromethane.

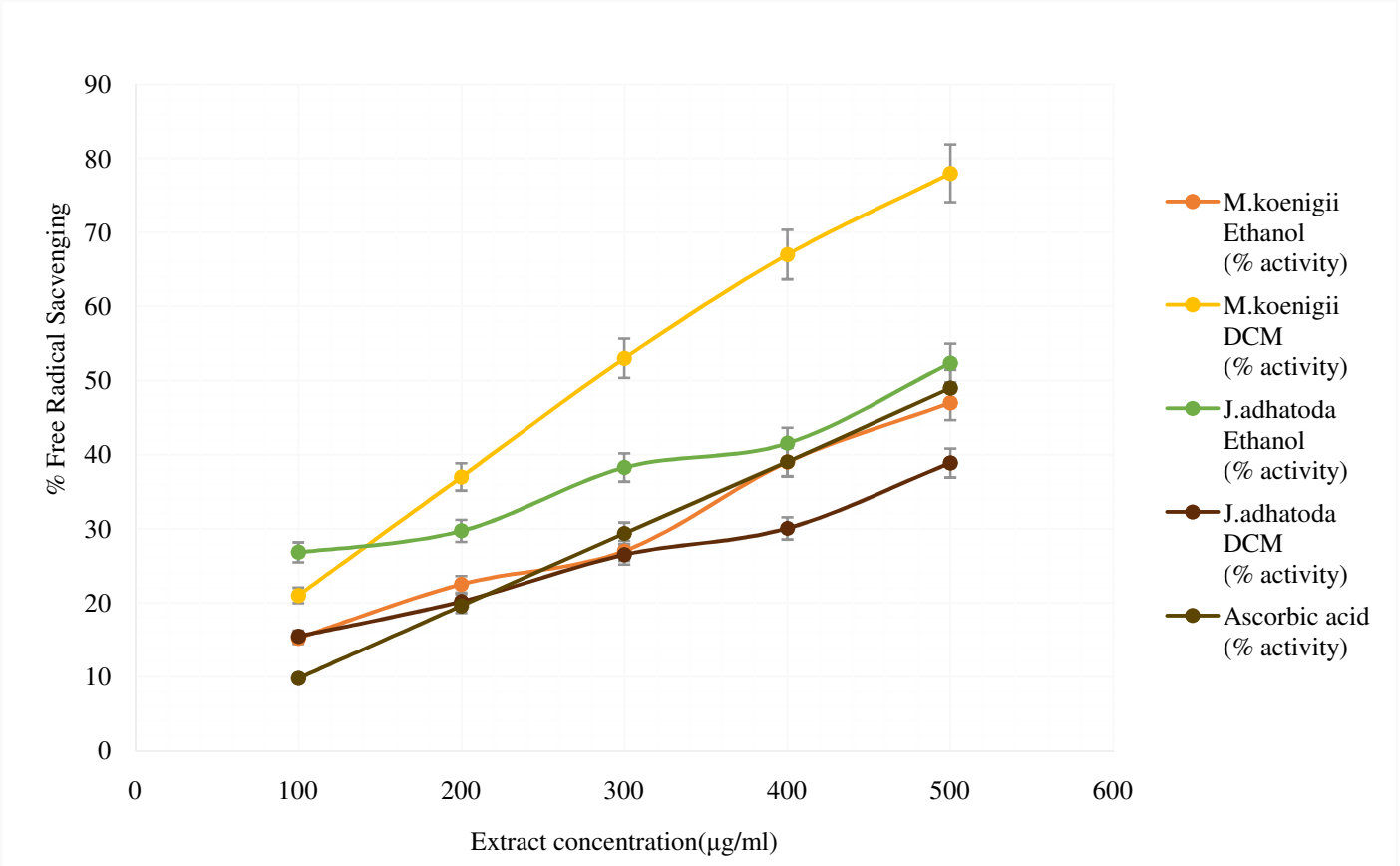

Figure-2: Graph depicting Percentage Free Radical Scavenging Activity of leaf extracts of *M.koenigii* and *J.adhatoda* with Ascorbic acid as Standard. 1 unit of X axis represents 100µg/ml of extract concentration. 1unit of Y axis represents 10% of % Free Radical Scavenging Activity.

Anti-diabetic activity: Ethanolic extract of *J.adhatoda* leaf showed best inhibition of Alpha-amylase enzyme activity followed by Ethanolic extract of *M.koenigii* leaf, DCM extract of *J.adhatoda* and lastly by DCM extract of *M.koenigii.* The standard drug, Acarbose showed the lowest inhibition of the alpha amylase enzyme. IC_{50} of all extracts and the standard for alpha-amylase activity has been calculated and shown in the last row of Table-4.

Table-4: Alpha amylase inhibition assay:- Percentage of Enzyme Inhibition with IC_{50} of leaf extracts of *J.adhatoda* and *M.koenigii.*

Conc. of extract (µg/ml)	*M.koenigii*		*J.adhatoda*		Acarbose (Standard) (% activity)
	Ethanol (% activity)	DCM (% activity)	Ethanol (% activity)	DCM (% activity)	
10	8.12±1.35	11.5±0.36	28±4.7	19.4±0.2	21.06±0.6
20	23.6±0.08	28.75±0.05	49±1.04	30.2±1.3	28.92±0.44
40	43.9±1.02	37.8±0.02	54±0.03	38.6±0.12	33.35±0.01
60	62.35±0.51	44.3±2.03	67±0.01	47.25±0.38	39.27±0.67
80	68.05±0.36	53.25±3.01	79±1.7	68.3±5.6	46.84±0.03
100	76.2±0.84	65.1±1.44	93.1±0.53	81.81±2.13	62.78±0.17
IC_{50}(µg/ml)	47.25±0.23	79.05±3.54	38.32±0.2	62.38±0.3	87.58±0.9

Acarbose was used as standard anti-diabetic drug; Values expressed as Mean±SD % α-amylase inhibition activity of *J.adhatoda* Extracts.

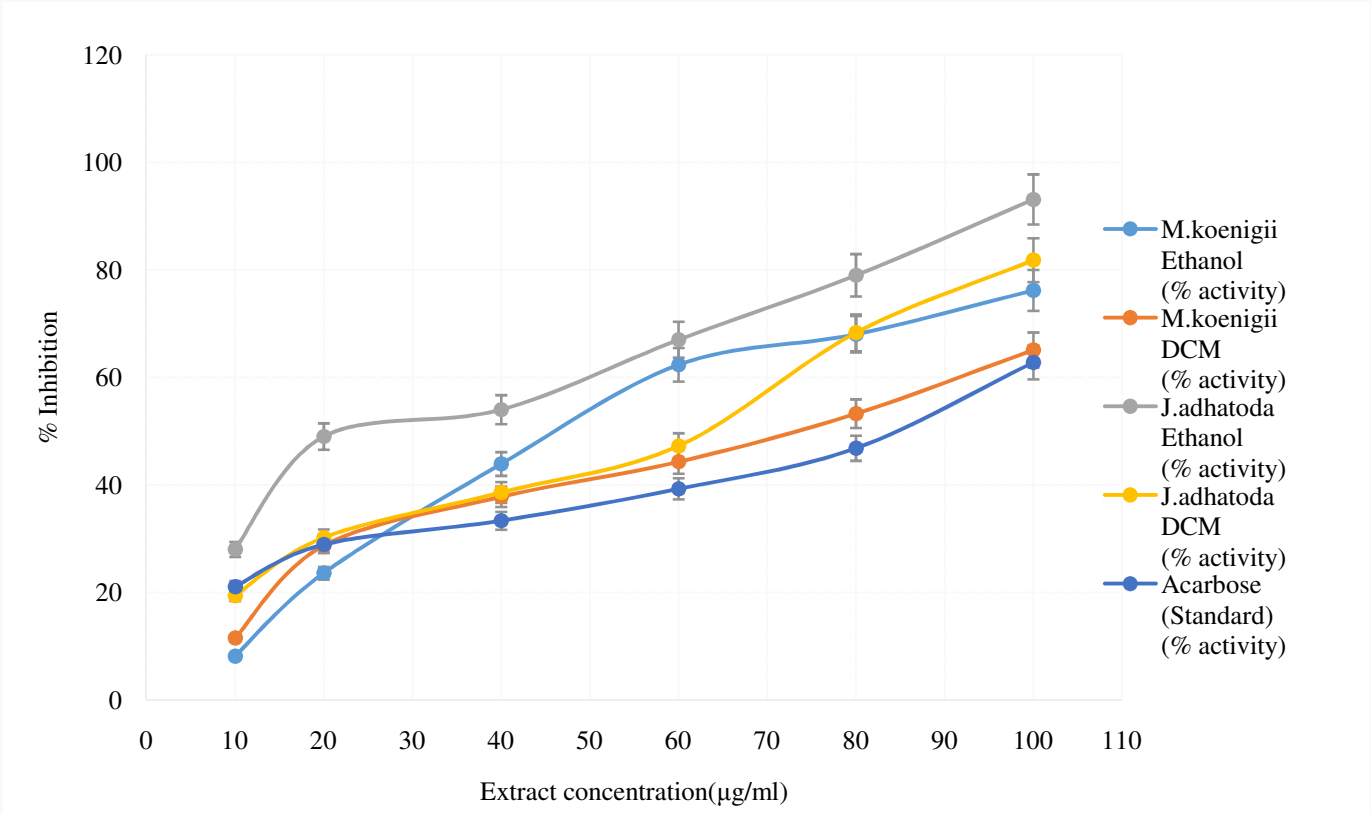

Figure-3: Graph depicting Percentage Alpha Amylase Inhibition Activity of leaf extracts of *M.koenigii* and *J.adhatoda* with Ascorbic acid as Standard. 1 unit of X axis represents 100µg/ml of extract concentration. 1unit of Y axis represents 20% of % Enzyme Inhibition.

Discussion: Our results indicate a good potential for anti-oxidant as well as anti-diabetic activity in both *Justicia adhatoda* and *Murraya koenigii*. However, *M.koenigii* Ethanolic leaf extract showed higher reducing power activity than all the other extracts in various solvents of both t. Although *J.adhatoda* Ethanolic leaf extract also gave good Reducing agent activity, but its activity was almost half of the Ethanol leaf extract of *M.koenigii*. Activity of DCM leaf extracts of both plants were almost equal and lowest as compared to Ethanolic leaf extracts of *M.koenigi*i and *J.adhatoda.*

The trends of activities shift when we looked at the Free radical scavenging activity. In free radical scavenging activity, DCM extract of *M.koenigii* showed best activity (IC_{50}=300±6.37 µg/ml) followed by Ethanolic extract of *J.adhatoda* (IC_{50}= 460±6.23µg/ml), Ethanolic extract of *M.koenigii* (IC_{50}= 520±4.21µg/ml) and DCM extract of *J.adhatoda* (IC_{50}= 600±10.84µg/ml). Activity of DCM extract of *M.koenigii* is significantly higher as compared to the other extracts. While, the activity of other extracts are lower and very much similar with each other. The activity of all extracts were more than Scavenging activity of Ascorbic acid as standard.

Anti-oxidant activity is generally directly related to the trends in Anti-diabetic activity. But our results are on the contrary. Where *M.koenigii* Ethanolic extract came out to be the best anti-oxidant, its anti-diabetic activity was lower than other plant extracts. Although Ethanolic leaf extract of *J.adhatoda* showed lower Reducing power activity than Ethanolic extract of *M.koenigii*, the in-vitro assay of α-amylase enzyme inhibition was found to be highest in Ethanolic extract of *J.adhatoda* (IC50=38.32±0.2µg/ml), which is followed by Ethanolic extract of *M.koenigii* (IC_{50}=47.25±0.23µg/ml), DCM extract of *J.adhatoda* (IC_{50}=62.38±0.3µg/ml) and *M.koenigii* (79.05±3.54 µg/ml). Acarbose, which is a well known anti-diabetic drug, showed lowest inhibition of α-amylase enzyme activity (IC_{50} = 87.58±0.9). The drug activity was found to be almost equal to DCM extract of *M.koenigii* leaf.

From the phytochemical (quantitative) analysis of all extracts, showed that Ethanolic leaf extracts of both plants have higher quantity of phytochemical level as compared to DCM leaf extracts. In Ethanolic leaf extracts of *M.koenigii*, all phytochemicals were found to be in almost equal quantity, having Tannin and Phenol content slightly higher, whereas Alkaloids and Flavonoids in lower quantity. However, DCM leaf extract of *M.koenigii* does not contain Alkaloids, but has high content of Phenols and Taninns. (Flavonoids are also present in traces) (Table-1). It can be inferred that Phenols and Taninns may play significant role towards their ability as reducing power agent. Other factors may also play important role as Anti-diabetic activity.

In *J.adhatoda* leaf extracts, Ethanolic extract contains exceptionally high amount of Alkaloids while, Phenols and Flavonoids are in moderate and Taninns in trace amount. Alkaloids in DCM extract was not found while contains Flavonoids in moderate quantity. DCM extract contains Taninns and Phenols in traces. In all assays conducted for determining Anti-oxidant and Anti-diabetic activity showed higher activity of Ethanolic extract of *J. adhatoda* leaf than DCM extract. After evaluating the results obtained in the assays and the quantitative analysis shows that Alkaloids play prominent role in anti-oxidant and anti-diabetic activity but not the sole factor. it can be observed that both extracts show almost equal Reducing power and slightly different Free radical scavenging activity. The difference is significant in anti-diabetic assay as IC_{50} of Ethanolic extract of *J.adhatoda* for anti-diabetic assay is 38.32±0.2µg/ml and IC_{50} of DCM extract of the same is 62.38±0.3µg/ml. From our results, it can be inferred that while alkaloids play significant role as anti-diabetic assay, Flavonoids are one of the prominent factor as anti-oxidant activity and have moderate activity as anti-oxidant.

Conclusion

Murraya koenigii and *Justicia adhatoda* leaf extracts show good *in-vitro* anti-oxidant and anti-diabetic activity. Although these plants are already in use for medicinal purpose in Ayurvedic and Unani system, they are not used in modern medicine. Hence they are classified as Alternate/Pseudo medicines. Our experimental results showed that *M.koenigii* Ethanolic extract has excellent anti-oxidant activity as well as good anti-diabetic activity. Whereas *J.adhatoda* Ethanolic extract came out to be the best in anti-diabetic activity, having moderate activity as an anti-oxidant too. DCM extracts of both plants show moderately good activity as anti-oxidant and anti-diabetic. From the phytochemical analysis it can be observed that, in *J.adhatoda* alkaloids could be the main potential compound for anti-diabetic activity but flavonoids, phenols and taninns also play certain role for the aforementioned properties. While in *M.koenigii* the properties are governed by phenols and taninns. Bioactivity guided fractionation of extracts and evaluation of their activity is currently underway.

Diabetes, although non-infectious, is currently emerging as one of the most fatal diseases worldwide. As plant based solution, *Murraya koenigii* and *Justicia adhatoda leaf extracts* emerge as potentially strong candidates that are needed to be extensively researched for their high antidiabetic and anti oxidant activities.

Acknowledgment

The present study is supported by the Department of Science and Technology (DST) grant (SB/SO/HS/186/2013). The authors are thankful to DST and University of Delhi, for giving them the opportunity to contribute to this research.

References

1. Olokoba A.B., Obateru O.A. and Olokoba L.B. (2012). Type 2 Diabetes Mellitus: A Review of Current Trends.

Oman Med. Journal, 27(4), 269-273. doi:10.5001/omj.2012.68.

2. WHO diabetes report (2018). Global report on Diabetes. retrieved from http://apps.who.int/iris/bitstream/10665/204871/1/9789241565257_eng.pdf

3. Mehra V., Mishra S., Gupta N. and Khatri M. (2018). Diabetes: The next epidemic. *Int. J. of. Life Sciences,* 6(2), 665-680.

4. US National Library of Medicine (2018). BYDUREON-exenatide. retrieved from https://dailymed.nlm.nih.gov/dailymed/drugInfo.cfm?setid=71fe88be-b4e6-4c2d-9cc3-8b1864467776

5. Gulfraz M., Ahmad A., Asad M.J., Afzal U., Imran M., Anwar P. and Qureshi R.U. (2011). Antidiabetic activities of leaves and root extracts of Justicia adhatoda Linn against alloxan induced diabetes in rats. *African Journal of Biotechnology*, 10(32), 6101-6106.

6. Ajay S., Rahul S., Sumit G., Paras M., Mishra A. and Gaurav A. (2011). Comprehensive review: *Murraya koenigii* Linn. *Asian J. Pharma. Life Sci.,* 1(4), 417-425.

7. Mehra V., Khatri M., Mishra S., Singh N., Sharma N., Goswami R. and Kothari P. (2018). Assessment of Anti-diabetic and Anti-oxidant activity of Murraya Koenigii extracts using in-vitro assays. *Journal of Biomedical and Therapeutic Sciences*, 5(1), 1-8.

8. Prabhavathi R.M., Prasad M.P. and Jayaramu M. (2016). Studies on Qualitative and Quantitative Phytochemical Analysis of *Cissus quadrangularis. Advances in Applied Science Research,* 7(4), 11-17.

9. Kulkarni A., Govindappa M., Channabasava Chandrappa C.P., Ramachandra Y.L. and Koka P.S. (2015). Phytochemical analysis of *Cassia fistula* and its in vitro antimicrobial, antioxidant and anti-inflammatory activities. *Advanced Medicinal Plant Research,* 3(1), 8-17.

10. Debiyi O. and Sofowora F.A. (1978). Pytochemical screening of medical plants. *Iloyidia,* 3, 234-246.

11. Garg V.K., Jain M., Sharma P.K. and Garg G.A.R.I.M.A. (2010). Anti-inflammatory activity of Spinacia oleracea. *International Journal of Pharma Professional's Research*, 1(1), 1-4.

12. Govindappa M., Naga S.S., Poojashri M.N., Sadananda T.S. and Chandrappa C.P. (2011). Antimicrobial, antioxidant and in vitro anti-inflammatory activity of ethanol extract and active phytochemical screening of *Wedelia trilobata* (L.), *Hitchc. Journal of Pharmacognosy and Phytotherepy,* 3(3), 43-51.

13. Madhu M., Sailaja V., Satyadev T.N.V.S.S. and Satyanarayana M.V. (2016). Quantitative phytochemical analysis of selected medicinal plant species by using various organic solvents. *Journal of Pharmacognosy and Phytochemistry,* 5(2), 25-29.

14. Harborne J.B. (1998). Phytochemical Methods. London: Chapman and Hall, 91.

15. Obadoni B.O. and Ochuko P.O. (2002). Phytochemical studies and comparative efficacy of the crude extracts of some haemostatic plants in Edo and Delta States of Nigeria. *Global Journal of pure and applied sciences*, 8(2), 203-208.

16. Liyana-Pathirana C.M. and Shahidi F. (2005). Antioxidant activity of commercial soft and hard wheat (Triticum aestivum L.) as affected by gastric pH conditions. *Journal of agricultural and food chemistry*, 53(7), 2433-2440.

17. Alam M.N., Bristi N.J. and Rafiquzzaman M. (2013). Review on in vivo and in vitro methods evaluation of antioxidant activity. *Saudi Pharmaceutical Journal*, 21(2), 143-152.

18. Phatak R.S. and Hendre A.S. (2014). Total antioxidant capacity (TAC) of fresh leaves of *Kalanchoe pinnata. Journal of Pharmacognosy and Phytochemistry,* 2(5), 32-35.

19. Brand-Williams W., Cuvelier M.E. and Berset C. (1995). Use of free radical method to evaluate antioxidant activity. *Lebens- Wiss Technol.,* 28*,* 25-30.

20. Conforti F., Statti G., Loizzo M.R., Sacchetti G., Poli F. and Menichini F. (2005). In Vitro antioxidant effect and inhibition of alpha-amylase of two varieties of *Amaranthus caudatus* seeds. *Biological and Pharmaceutical Bulletin,* 28(6)*,* 1098-1102.