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A Study on physical parameters and Quantitative analysis of alcoholic and hydro alcoholic extraction of *Excoecaria agallocha L* in albino rats

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ABSTRACT

The present study was designed to investigate the effect of different extracts of *Excoecaria agallocha L*. against diabetes mellitus and its related complications. Diabetes was induced by intraperitoneal administration of streptozotocin STZ (60 mg/kg) for the development of diabetic neuropathy. Treatment with different extracts of *Excoecaria agallocha L* significantly attenuated elevated thermal and mechanical Hyperalgesia. Elevated tactile allodynia assessed using flexible Von Frey filaments was also reversed by extracts. Treatment with both the extracts significantly attenuated the parameters of oxidative stress in sciatic nerve of diabetic neuropathic rats. These findings suggested that the *Excoecaria agallocha L*. may be used to manage the diabetes mellitus and its related complications such as diabetic neuropathy.

Keywords: *Excoecaria agallocha*, streptozotocin STZ etc.

INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by a loss of glucose homeostasis with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. Without enough insulin, the cells cannot absorb sufficient glucose from the blood;

hence blood glucose levels increase, and result as hyperglycemia. If the blood glucose level remains high over a long period, it can result in long-term damage to organs, such as kidneys, liver, eyes, nerves, heart and blood vessels. Complications in some of these organs can lead to death also. Diabetes mellitus (sometimes called "sugar diabetes") is a condition that occurs when the body can't use glucose (a type of sugar) normally.

Glucose is the main source of energy for the body's cells. The levels of glucose in the blood are controlled by a hormone called insulin, which is made by the pancreas.

About plant introduction

Excoecaria agallocha L.

Excoecaria agallocha L. (Euphorbiaceae) is an ancient mangrove tree also known as Blinding tree; Tillai, Kampetti (Tamil); Tilla, Tella and Chilla (Telugu); Telakiriya, Talia (Singhalese). It is widely distributed in the mangrove region of Pichavaram, Indian coastal regions, Australia from Northern New South Wales, along the northern coast line of Western Australia (Ajithkumar *et al*; 2008 & Ding *et al*; 2010).

Morphological characteristics

Excoecaria agallocha is a deciduous and dioecious tree of about 15 meter height with abundance of latex (Duke, 2006.) The latex is toxic in nature and may create blindness and blisters over skin. The stem bark is greenish in colour with lenticellate and fissures

arranged vertically. The root part remains conjugated with each other. The leaves are orange-red colored, alternate and ovate elliptic, arranged oppositely with glands. The flowers are yellowish green in color, larger in males than the females, with specific odor. The fruit is encapsulated, globose with 3lobes, containing black colored numerous seeds. The pollination occurs by the insect (Li *et al*; 2010).

Traditional Values

The *Excoecaria agallocha* is considered as a sacred plant by the local community of the tamilanadu, and being used for the worship (Kali murthi and Selvaraj; 2016). Traditionally, the *Excoecaria agallocha* is being used in the various regions of Tamilnadu to treat various ailments like sores, ulcers, as a laxative and purgative. The plant part especially leaves are being used as emetic agent due to its bitter taste. The fumes of bark are also used to treat leprosy (Lu *et al*; 2010). On the other hand *Excoecaria agallocha* leaves are also being used against, snakebites, hemorrhagic fever, rheumatoid etc. The larvicidal, and mosquito repellents property have also documented traditionally (Selvaraj *et al*; 1995).



Fig 1: Leaves of the *Excoecaria agallocha* L.

Chemical constituents

A number of primary and secondary metabolites have been well reported in various parts of *Excoecaria agallocha* like, leaves, bark, stem, root and flowers. The plant is abundant in presence of terpenoids (especially, diterpenoids,) along with triterpenoids,

phenols, flavones, sterols (phytosterol), tannins, carbohydrates, glycosides and amino acids. (Wang *et al*; 2012)(Yin *et al*; 2008).

MATERIALS AND METHODS

Quantative Analysis

Estimation of Total Phenolic Content

According to the Folin–Ciocalteu method, the total phenolic (soluble) content was estimated by using the Folin–Ciocalteu reagent. This method based on the oxidation reaction. Gallic acid was used as standard reagent in this procedure Liu et al. (2013). Extract

solution (1.0 g/ml) was taken in the flask and then dilution of extract was made up to 46 ml with distilled water. After dilution, Folin–Ciocalteu reagent (1 ml) was added and mixed. After proper mixing, the solution was stand for 3 minutes. Further sodium carbonate was mixed into the above mixture solution and allowed to stand for 180 minutes by occasional shaking. Blue color developed was then noted at 760 nm. Phenolic compounds in the extract were determined as μg of Gallic acid equivalent.

Table 1: The absorbance of Standard Compound (Gallic acid)

S.No	Concentration (ug/ml)	Absorbance of STD(Gallic Acid)
1	10	0.142±0.014
2	20	0.292±0.011
3	30	0.324±0.017
4	40	0.469±0.021
5	50	0.576±0.016
6	60	0.668±0.018
7	70	0.769±0.023
8	80	0.828±0.034
9	90	0.934±0.023
10	100	1.078±0.026

Values are mean \pm SD of three replicates (n=3)

Table 2: The absorbance of alcoholic extract of *Excoecaria agallocha* leaves

S.No	Concentration (ug/ml)	Absorbance
1	50	0.09±0.002
2	100	0.178±0.003
3	200	0.298±0.005
4	300	0.484±0.006
5	400	0.580±0.004
6	500	0.705±0.003

Values are mean \pm SD of three replicates (n=3)

Table 3: The absorbance of Hydro- alcoholic extract of *Excoecaria agallocha* leaves

S. No	Concentrations (ug/ml)	Absorbance
1	50	0.09±0.011
2	100	0.162±0.018
3	200	0.303±0.028
4	300	0.478±0.034
5	400	0.589±0.029
6	500	0.711±0.033

Values are mean \pm SD of three replicates (n=3)

ESTIMATION OF TOTAL FLAVONOID CONTENT

Total flavonoid content was measured with the aluminium chloride colorimetric assay. 1ml of aliquots and 1ml standard Quercetin solution (30,40,50,60,70,80,90,100 µg/ml) was positioned into test tubes and 4ml of distilled water and 0.3 ml of 5 % sodium nitrite solution was added into each. After 5

minutes, 0.3 ml of 10 % aluminum chloride was added. At 6th minute, 2 ml of 1 M sodium hydroxide was added. Finally, volume was making up to 10 ml with distilled water and mix well. Orange yellowish color was developed. The absorbance was measured at 510 nm spectrophotometer using UV-visible Jasco V-630 instrument. The blank was performed using distilled water. Quercetin was used as standard. The samples were performed in triplicates. The calibration curve was plotted using standard Quercetin.

Table 4: The absorbance of Standard Compound (Quercetin)

S.No	Concentrations (ug/ml)	Absorbance of STD (Quercetin)
1	30	0.293±0.012
2	40	0.347±0.016
3	50	0.491±0.019
4	60	0.529±0.021
5	70	0.612±0.023
6	80	0.697±0.017
7	90	0.784±0.019
8	100	0.996±0.012

Values are mean ±SD of three replicates (n=3)

Table 5: The absorbance of alcoholic extract of *Excoecaria agallocha* leaves

S.No	Concentrations (ug/ml)	Absorbance
1	50	0.232±0.012
2	100	0.295±0.019
3	200	0.391±0.023
4	300	0.489±0.012
5	400	0.590±0.015
6	500	0.727±0.021

Values are mean ±SD of three replicates (n=3)

Table 6: The absorbance of Hydro- alcoholic extract of *Excoecaria agallocha* leaves

S.No	Concentrations (ug/ml)	Absorbance
1	50	0.216±0.013
2	100	0.322±0.018
3	200	0.398±0.021
4	300	0.472±0.016
5	400	0.589±0.023
6	500	0.673±0.014

Values are mean ±SD of three replicates (n=3)

STATISTICAL ANALYSIS

Statistical analysis was performed using Graph Pad Prism 6. Values were expressed as mean \pm SEM and one way analysis of variance (ANOVA) was used for statistical analysis. One way ANOVA followed by Tukey's multiple test; **a** vs control; **b** vs Diabetic control; **c** vs 100 mg/kg (LD); **d** vs 200 mg/kg (MD); **e** vs Standard. * $p < 0.001$, # $p < 0.01$, † $p < 0.05$.

RESULTS

Extraction

Size reduced powder of leaves of *Excoecaria agallocha* were extracted separately by Soxhlet extraction technique with alcohol and hydro-alcohol (70%). Extractive yield from respective solvents is mentioned in **table 6**.

Percentage yield of the extracts

The percentage yield of the collected extracts was calculated accordingly and was found as mentioned in **table no.7**

$$\text{Percentage yield} = \frac{\text{Weight of extracts obtained}}{\text{Weight of crude extracts}} \times 100$$

Table 7: Percentage yield of the collected extracts

S. no	Extract	Weight taken (Grams)	Percentage yield
1	Alcoholic extract of <i>Excoecaria agallocha</i>	500	52%
2	Hydro alcoholic <i>Excoecaria agallocha</i>	500	38%

Result of Phytochemical Screening

The Preliminary phytochemical investigation revealed the presence of various phytoconstituents in extracts of *Excoecaria agallocha* leaves. The results of phytochemical screening were found as given in table below. The extracts of *Excoecaria agallocha* leaves were analyzed for the presence of present phytoconstituents, although the plant material was collected from pollutant free area and hygienic. The

phytochemical screening was performed to determine the presence of carbohydrates, alkaloids, glycosides, phenols, fatty material and nitrogenous compounds i.e. proteins and amino acids. The result revealed that both the extracts were rich in terms of alkaloids, carbohydrates, glycosides, flavonoids and phenols. Yet the extracts were rich with steroidal moiety. The intensity of phenolic and flavonoid determining inference were high.

Table 8: Result of Preliminary phytochemical screening of various extract of *Excoecaria agallocha* leaves

Phytochemical	Alcoholic extract of <i>Excoecaria agallocha</i>	Hydro-alcoholic extract of <i>Excoecaria agallocha</i>
Carbohydrates	+	+
Glycosides	+	+
Flavonoids	+	+
Saponins	-	+
Alkaloids	+	+
Proteins and amino acids	-	-

Phenol and phenolic compounds	+	+
Phytosterol	+	+

Note: + sign indicate the presence; - sign indicate the absence

Physical Parameters

The physical parameters of extracts from leaves of *Excoecaria agallocha* showed in **table 9**.

Table 9: Physical Parameters of Leaves of *Excoecaria agallocha*

S. no.	Physical parameter	% (with reference to air dried drug)
1.	Alcohol soluble extractive	6
2.	Water soluble extractive	8.5
3.	Ether soluble extractive	8.3
4.	Chloroform soluble extractive	2.5
5.	Total ash	11
6.	Acid insoluble ash	4
7.	Water soluble ash	7
8.	Loss on drying	15

TOTAL PHENOL CONTENT

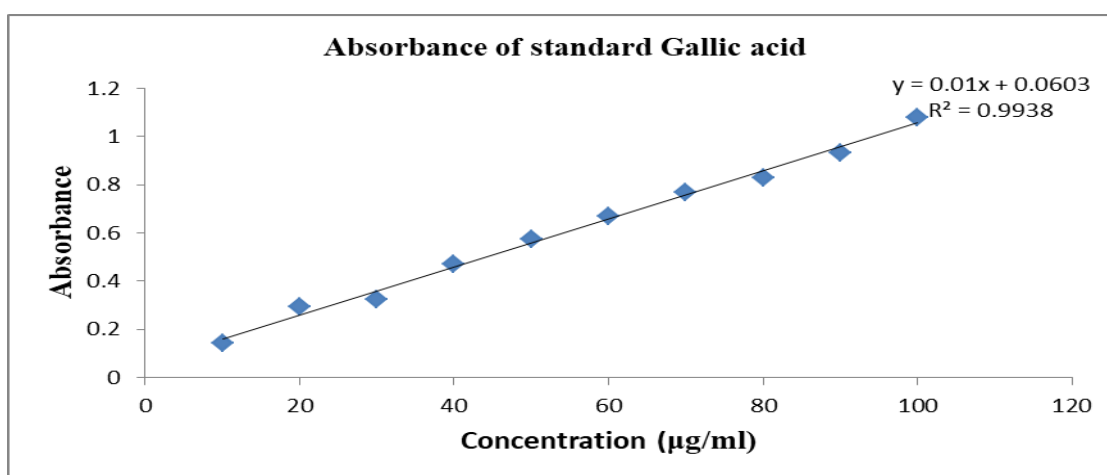


Fig 2: Standard curve of Gallic acid

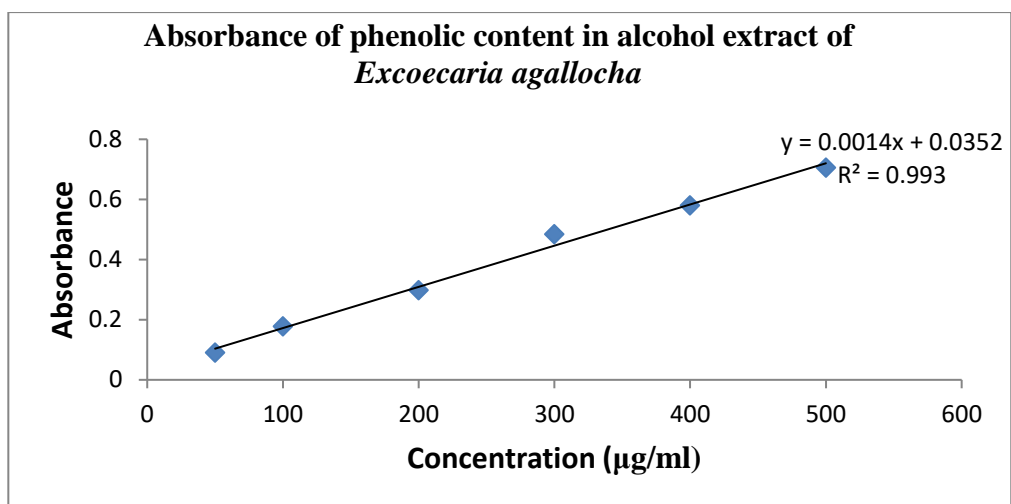


Fig 3: Total phenol content in alcoholic extract of *Excoecaria agallocha* leaves

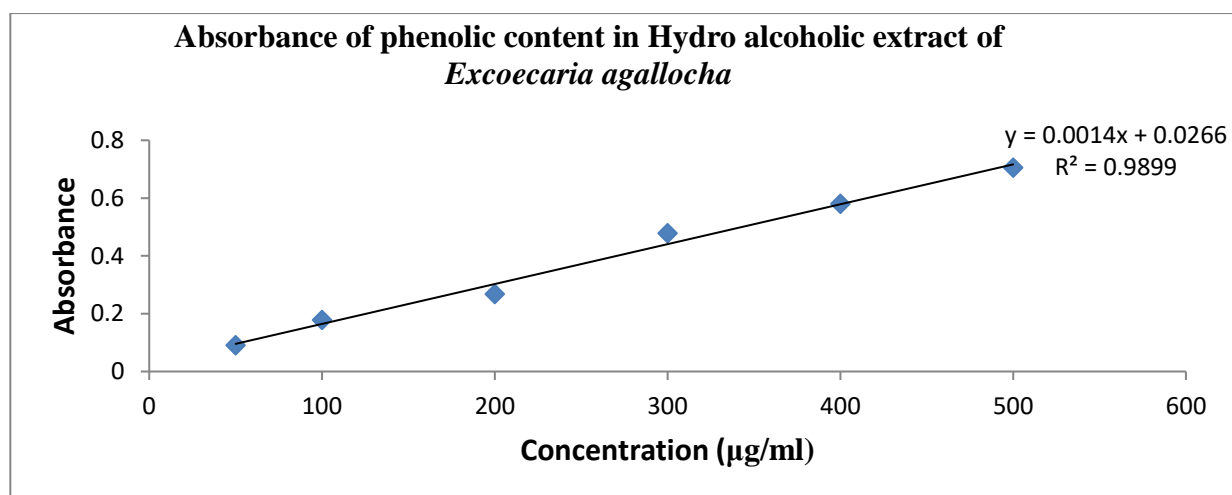


Fig 4: Total phenol content in Hydro-alcoholic extract of *Excoecaria agallocha* leaves

Table 10: Total phenol content in alcoholic and Hydro-alcoholic extract of *Excoecaria agallocha* leaves

Sr. No	Extracts	Total phenolic content (mg /g Gallic acid equivalent)
1	Alcoholic extract of <i>Excoecaria agallocha</i> leaves	2.71
2	Hydro-alcoholic extract of <i>Excoecaria agallocha</i> leaves	3.76

Total Flavonoid Contents

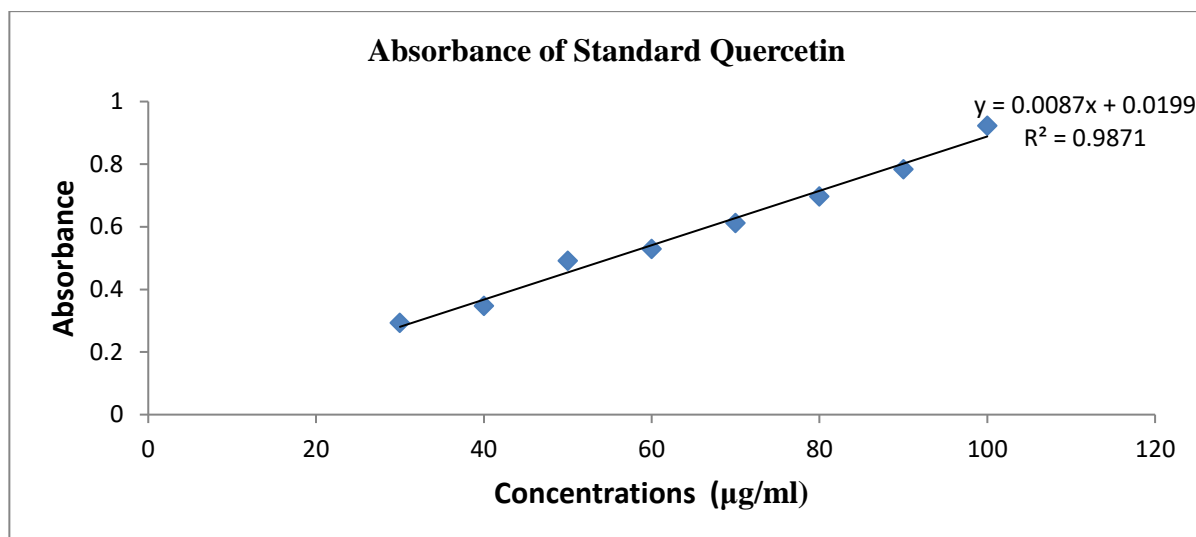


Fig 5: Standard curve of Quercetin

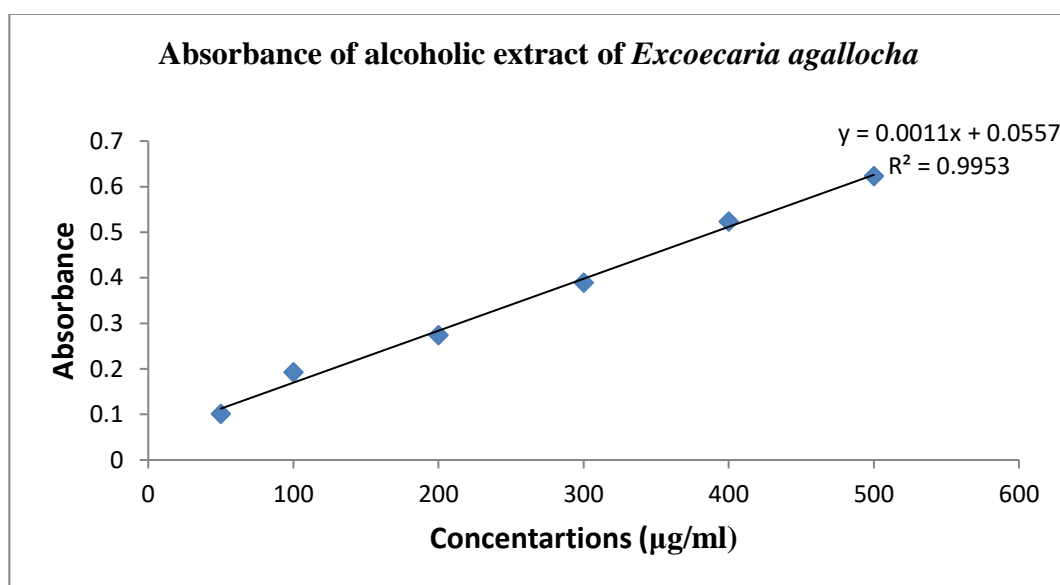


Fig 6: Total flavonoid content in alcoholic extract of *Excoecaria agallocha* leaves

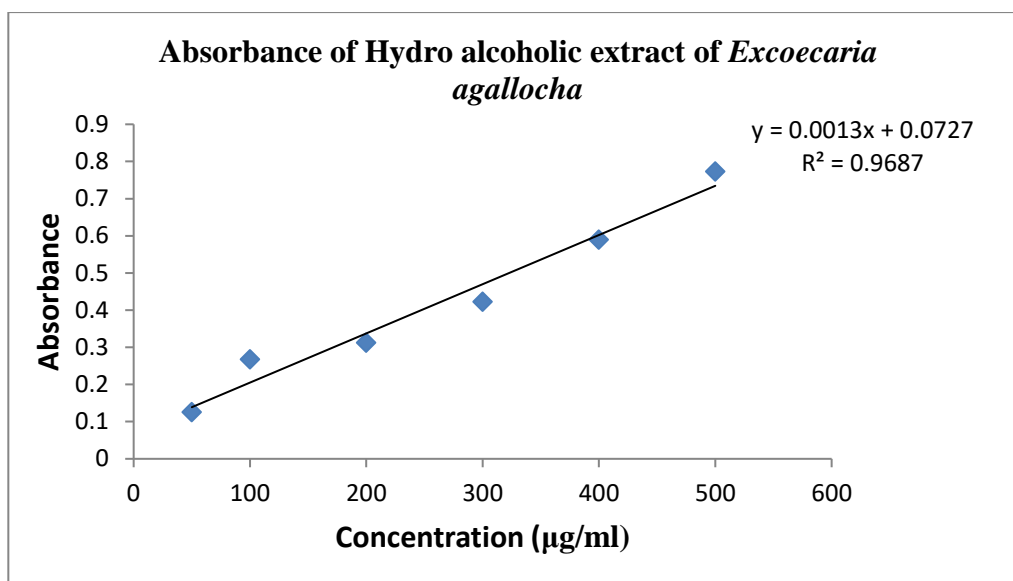


Fig 7: Total flavonoid content in hydro-alcoholic extract of *Excoecaria agallocha* leaves

Table 11: Total flavonoid content in alcoholic and Hydro-alcoholic extract of *Excoecaria agallocha* leaves

Sr. No	Extracts	Total flavonoid content (mg /g Quercetin equivalent)
1	Alcoholic extract of <i>Excoecaria agallocha</i> leaves	5.14
2	Hydro-alcoholic extract of <i>Excoecaria agallocha</i> leaves	7.57

DISCUSSION

Diabetes mellitus is a group of longstanding disorders manifested by hyperglycemia. Etiology of diabetes is related to defects in insulin secretion and/or insulin action that leading to hyperglycemia. It has been revealed that chronic hyperglycemia, the hallmark of DM, leads to protein glycation and formation of advanced glycation products (AGE), which contribute to both microvascular and macrovascular complications. These complications affect the entire body specially, the eyes, nerves, kidneys, livers, blood vessels, heart and contribute to morbidity and mortality of diabetes. In addition, hyperglycemia can lead to high production of reactive oxygen species (ROS) and simultaneous reduction of the antioxidant defence mechanisms, which can cause oxidative stress. Therefore, hyperglycemia induced oxidative stress due to the cellular function disruption and cellular damage has a crucial role in the development and progression

of diabetic complications. Diabetic nephropathy as a micro vascular complication is responsible for about 30-40% of all end-stage renal disease cases. When hyperglycemia is maintained for a long time, nephropathy occurs due to the multiple cellular mechanisms including, activation of protein kinase C (PKC) pathway, cytokines production, enhanced polyol pathway, increased formation of advanced glycation end products (AGE), increased oxidative stress and hexosamine pathway (Ajebli et al; 2019).

The present study, effect of *Excoecaria agallocha* leaves extraction on diabetes mellitus and its associated complications was investigated on diabetic rats after literature survey the plant was procured in Andhra Pradesh.

The study result reveals that alcoholic and hydro alcoholic extracts were rich in terms of alkaloids, carbohydrates, glycosides, flavonoids and phenols are responsible for pharmacological actions in this study. As per the comparative study in quantitative analysis for

total phenolic content in alcoholic and hydro alcoholic extractions of *Excoecaria agallocha* leaves as resulted hydro alcoholic content is rich. As per the comparative study in quantitative analysis for total flavonoids content in alcoholic and hydro alcoholic extractions of *Excoecaria agallocha* leaves as resulted hydro alcoholic content is rich. As per the comparative study in quantitative analysis for total antioxidant content in alcoholic and hydro alcoholic extractions *Excoecaria agallocha* leaves as resulted hydro alcoholic content is rich.

REFERENCES

1. Alkan EE, Celik I. The therapeutics effects and toxic risk of *Heracleum persicum* Desf. extract on streptozotocin-induced diabetic rats. *Toxicol Rep.* 2018;5:919-26. doi: 10.1016/j.toxrep.2018.08.004, PMID 30225197.
2. Al-Malki AL, Barbour EK, Abulnaja KO, Moselhy SS. Management of hyperglycaemia by ethyl acetate extract of *Balanites aegyptiaca* (Desert Date). *Molecules.* 2015;20(8):14425-34. doi: 10.3390/molecules200814425, PMID 26262605.
3. Althunibat OY, Al Hroob AM, Abukhalil MH, Germoush MO, Bin-Jumah M, Mahmoud AM. Fisetin ameliorates oxidative stress, inflammation and apoptosis in diabetic cardiomyopathy. *Life Sci.* 2019;221:83-92. doi: 10.1016/j.lfs.2019.02.017, PMID 30742869.
4. Anchi P, Khurana A, Swain D, Samanthula G, Godugu C. Dramatic improvement in pharmacokinetic and pharmacodynamic effects of sustain release curcumin microparticles demonstrated in experimental type 1 diabetes model. *Eur J Pharm Sci.* 2019;130:200-14. doi: 10.1016/j.ejps.2019.02.002, PMID 30731237.
5. Anil Kumar V, Ammani K, Jobina R, Parasuraman P, Siddhardha B. Larvicidal activity of green synthesized silver nanoparticles using *Excoecaria agallocha* L. (Euphorbiaceae) leaf extract against *Aedes aegypti*. *IET Nanobiotechnology.* 2016;10(6):382-8. doi: 10.1049/iet-nbt.2015.0101, PMID 27906138.
6. Anjaneyulu AS, Rao VL, Sreedhar K. Agallochins J-L, new isopimarane diterpenoids from *Excoecaria agallocha* L. *Nat Prod Res.* 2003;17(1):27-32. doi: 10.1080/1057563021000027975, PMID 12674139.
7. Bai W, Zhang C, Chen H. Transcriptomic analysis of *Momordica charantia* polysaccharide on streptozotocin-induced diabetic rats. *Gene.* 2018;675:208-16. doi: 10.1016/j.gene.2018.06.106, PMID 30180967.
8. Balakrishnan BB, Krishnasamy K, Choi KC. *Moringa concanensis* Nimmo ameliorates hyperglycemia in 3T3-L1 adipocytes by up regulating PPAR- gamma, C/EBP-alpha via Akt signaling pathway and STZ-induced diabetic rats. *Biomed Pharmacother.* 2018;103:719-28. doi: 10.1016/j.biopha.2018.04.047.
9. Banerjee K, Das S, Choudhury P, Ghosh S, Baral R, Choudhuri SK. A novel approach of synthesizing and evaluating the anticancer potential of silver oxide nanoparticles in vitro. *Chemotherapy.* 2017;62(5):279-89. doi: 10.1159/000453446, PMID 28490010.
10. Barbari GR, Dorkoosh F, Amini M, Bahari Javan N, Sharifzadeh M, Atyabi F, Balalaie S, Rafiee Tehrani N, Rafiee Tehrani M. Synthesis and characterization of a novel peptide-grafted Cs and evaluation of its nanoparticles for the oral delivery of insulin, in vitro, and in vivo study. *Int J Nanomedicine.* 2018;13:5127-38. doi: 10.2147/IJN.S161240, PMID 30233176.
11. Chang B, Jin C, Zhang W, Kong L, Yang JH, Lian FM, Li QF, Yu B, Liu WK, Yang LL, Zhao P, Zhen Z. *Euonymus alatus* in the treatment of diabetic nephropathy in rats. *Am J Chin Med.* 2012;40(6):1177-87. doi: 10.1142/S0192415X12500875, PMID 23227790.
12. Chang X, Lu K, Wang L, Lv M, Fu W. *Astragalus* polysaccharide protects diabetic cardiomyopathy by activating NRG1/ErbB pathway. *BioSci Trends.* 2018;12(2):149-56. doi: 10.5582/bst.2018.01027, PMID 29607874.

CONCLUSION

The Results obtained in the present study suggested that *Excoecaria agallocha* L. have higher amount of phenols, which are known to scavenge free radicals. Further, the study reflects the ability of these plants to inhibit the formation of AGEs and sorbitol accumulation and inhibition of enzyme ALR which has been recognized as important strategy in the attenuation of long term.

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