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EVALUATION OF ANTIOXIDANT, ANTI-LIPID PEROXIDATION, AND ANTI-DIABETIC PROPERTIES OF ETHANOL EXTRACT OF ZAPPANIA URTICIFOLIA (EEZU): A PHYTOCHEMICAL AND ENZYMATIC STUDY

Sreenadh B*, Tejasri A, Pravallika B, Eswar G, Hemanth K, Sireesha M

Department of Pharmaceutical Analysis, Jagan's College of Pharmacy, Nellore-524346

ABSTRACT

The study aimed to investigate the antioxidant, anti-lipid peroxidation, and anti-diabetic properties of the ethanol extract of Zappania urticifolia (EEZU). Fresh plant material of Zappania urticifolia was collected and authenticated, followed by ethanol extraction to obtain EEZU. Phytochemical analysis revealed the presence of alkaloids, flavonoids, and tannins in the extract. The anti-diabetic potential of EEZU was evaluated through α-amylase and α-glucosidase inhibition assays. The results indicated that α-amylase inhibitory activity of EEZU was concentration-dependent, with the IC50 value determining the concentration required for 50% inhibition. Comparison with the standard drug, Acarbose, demonstrated the effectiveness of EEZU, particularly at higher concentrations. These findings align with existing literature on the medicinal potential of Zappania urticifolia, suggesting its value as a natural therapeutic resource. The study provides a comprehensive understanding of the potential therapeutic benefits of EEZU, highlighting its relevance in natural medicine for managing oxidative stress and diabetes.

Key words: Zappania urticifolia, anti-diabetic, antioxidant, Herbal remedy, Phytoconstituents.

Corresponding Author: Sreenadh B - E-mail:sreenadhbikki1156@gmail.com

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INTRODUCTION

The use of natural products with therapeutic properties is as ancient as human civilization and, for a long time, mineral, plant and animal products were the main sources of drugs. However, even if we only consider the impact of the discovery of the penicillin, obtained from micro-organisms, on the development of anti-infection therapy, the importance of natural products is clearly enormous. The vast majority of these cannot yet be synthesized economically and are still obtained from wild or cultivated plants. Natural compounds can be lead compounds, allowing the design and rational planning of new drugs, biomimetic synthesis development and the discovery of new therapeutic properties not yet attributed to known compounds.

Plants have been the basis of different traditional medicinal systems throughout the world and continue to provide mankind with new remedies. Natural products have served as a major source of drugs for centuries and about half of the pharmaceuticals in use today are derived from natural products. The demand for plant based medicines, health products, pharmaceuticals, food supplement, cosmetics etc., are increasing in both developing and developed countries, due to the growing recognition that the natural products are non-toxic, have less side effects and easily available at affordable prices. Different strategies will result in an herbal medicine or in an isolated active compound. Anti-oxidants are substances capable to end up free radicals and prevent them from causing cell damage. Free radicals are capable causing a

wide number of health problems which include cancer, heart diseases, and gastric problems etc.

Human body system is enriched with natural antioxidants and can prevent the onset of diseases and treat diseases caused due to free-radical mediated oxidative stress. Moreover, knowledge of the main pharmacologically active plant compounds is an essential requirement for the standardization and analysis of formulations. Diabetes mellitus is a chronic metabolic disorder, characterized by elevated blood glucose levels and disturbances in carbohydrates, fats and protein metabolism. IDDM is the most severe form of diabetes, occurs due to complete loss of pancreatic β-islet cells and hence there is insulin deficiency in which shots of insulin are necessary on a daily basis. The islets of langerhans are the endocrine component of the pancreas and they constitute 1% of the total pancreatic mass (Joy pp *et al.*,1998). It is synthesized in the pancreatic beta-cells. It is synthesized initially as polypeptide precursor-preproinsulin. Diagnosis of diabetes can be based on the presence of suggestive symptoms together with lab results that support the specific diagnosis.

MATERIALS AND METHODS

Collection of specimens

Fresh leaves of *Zappania urticifolia* was collected local area in Nellore, Andhra Pradesh from during February, 2024. The taxonomical identification and authentication of the plant was done by Dr. G. Avinash Kumar, Professor, Sun Institute of Pharmaceutical Education and Research, Nellore (Kamboj VP *et al.,* 2000 & Mukherjee PK. *et al.,* 2008).

Phytochemical Screening

Materials

Petroleum ether, chloroform, methanol, distilled water, hydrochloric acid, sulphuric acid, acetic anhydride, sodium nitroprusside, pyridine, potassium hydroxide, phenolphthalein, ferric chloride, gelatin, sodium chloride, lead acetate, bromine, magnesium, benzene, silica gel, Mayer's reagent Dragendroff's reagent, Wagner's reagent, Hager's reagent, alcoholic α-Naphthol, Fehling's reagent, Benedict's reagent, Millon's reagent, Biuret reagent, Ninhydrin solution.

Extraction

About 1kg of the powdered leaf material was successively extracted with solvents like petroleum ether, chloroform, Benzene and ethanol in a Soxhlet apparatus. The extracts were concentrated and traces of the solvent were completely removed under reduced pressure and stored in vacuum desiccators for further use. Aqueous extract was prepared by macerating the leaf powder in double distilled water. The extract was concentrated in water bath and stored in desiccators.

Preliminary Phytochemical Screening (Sen.S, *et al.,* 2012 & Verma .S *et al*, 2008**)**

The concentrated extracts were subjected to chemical tests as per the methods mentioned below for the identification of the various constituents as per the standard procedures given by Kokate and Trease and Evans.

Detection of Alkaloids

Small portions of solvent-free extracts were stirred separately with a few drops of dilute hydrochloric acid and filtered. The filtrate was tested with various alkaloidal reagents.

a. Mayer's test: Filtrates were treated with potassium mercuric iodide (Mayer's reagent) and the formation of cream coloured precipitate was indicates the presence of alkaloids.

b. Dragendroff's test: Filtrates were treated with potassium bismuth iodide (Dragendroff's reagent) and formation of reddish brown precipitate was indicates the presence of alkaloids.

c. Wagner's test: Filtrates were treated with solution of iodine in potassium iodide (Wagner's reagent) and formation of brown precipitate was indicates the presence of alkaloids.

d. Hager's test: Filtrates were treated with a saturated solution of picric acid (Hager's reagent) and formation of yellow precipitate was indicates the presence of alkaloids.

Determination of Total phenolic content (Thomas J *et al.,* 1997**;** Sen S, *et al*., 2009)

Following the literature and the results of preliminary phyto chemical screening for plant to contain polyphenols, it was tested to estimate the total phenolic content. It was measured colorimetrically using quercetin and FC (Folin Ciocault's) reagent.

Standard curve of quercetin

1mg of quercetin was weighed and dissolved in 100ml of distilled water and successive dilutions were made to make up the concentrations 2,4,6,8 and 10 µg/ml. A volume from above aliquots was taken and mixed with 1.25ml of FC reagent. It was left for 5 mins. Then 2.5ml of 20% sodium carbonate was added and it was let to react for 30 min then the volume was made upto 10ml. Then the absorbance was measured at 765 nm. The calibration curve was drawn plotting the absorbance and concentrations.

Sample preparation

0.5g of extract was weighed and dissolved in 100ml of water. From this 0.1ml was taken into 10ml standard flask and 1.25ml of FC reagent was added and let to react for 5 min. then 2.5ml of 20% sodium carbonate was added and the volume was made upto 10ml. it was kept for 30 min for complete reaction. (Sen S, *et al*., 2010) Now the absorbance was measured at 765 nm. Total phenolic content was calculated from the calibration curve of

quercetin and the value was expressed in quercetin equivalents.

RESULTS

The phytochemical analysis helps in formulating pharmacopoeial standards. The chief phytochemicals present in the different extracts of *Zappania urticifolia* were flavonoids, polyphenols, alkaloids, triterpenoids, steroids, tannins, carbohydrates, saponins and aminoacids.

In the investigation of *Zappania urticifolia*, a comprehensive phytochemical analysis was conducted on various solvent extracts. The pet ether extract exhibited a percentage yield of 5.38% (w/w) with a total phenol content of 31.22±2.38. Similarly, the benzene extract demonstrated a percentage yield of 2.56% (w/w) and a total phenol content of 22.28±2.18. Notably, the chloroform extract displayed a higher percentage yield at 10.25% (w/w), although the total phenol content was measured at 18.05±3.42. (Samy RP *et al*., 2008) The ethanol extract, with a substantial percentage yield of 26.64% (w/w), exhibited the highest total phenol content among the extracts, recording 255.67±19.56. The water extract, with a percentage yield of 20.18% (w/w), displayed a total phenol content of 183.93±12.17. (Sen S, *et al.,* 2010 & Sen S, *et al.,* 2010) These results underscore the variation in both yield and total phenol content across different solvent extracts, with the ethanol extract standing out for its notably high phenolic content, suggesting its efficacy in extracting bioactive compounds from *Zappania urticifolia*.

In-vitro α-amylase inhibitory activity

In the investigation of in-vitro α-amylase inhibitory activity, the ethanol extract of *Zappania urticifolia* (EEZU) exhibited concentration-dependent inhibition. At 100µg/ml, EEZU showed a % α-amylase inhibition of 8.11±1.76 with an IC50 value of 268.89 µg/ml. As the concentration increased to 200µg/ml, the % α-amylase inhibition rose to 26.65±3.16. Further increases in concentration (400µg/ml, 800µg/ml, and 1000µg/ml) led to escalating inhibitory activities, reaching 43.65±1.32, 61.188±2.71, and 77.43±1.52, respectively. (Tiwari AK *et al.,* 2001)

Comparatively, the standard drug (Acarbose) demonstrated significant α-amylase inhibitory activity at lower concentrations. At 5µg/ml, Acarbose exhibited a % α-amylase inhibition of 14.23±1.44 with an IC50 value of 28.45µg/ml. As the concentration increased (10µg/ml, 20µg/ml, 40µg/ml, and 50µg/ml), the α-amylase inhibitory activity of Acarbose escalated, reaching 28.45±1.44, 48.71±0.68, 75.32±1.70, and 88.81±1.85, respectively. These findings suggest the potential of EEZU in inhibiting α-amylase activity, with notable concentration-dependent effects. The comparison with Acarbose highlights the effectiveness of both substances in inhibiting α-amylase, with Acarbose demonstrating higher activity at lower concentrations.

In-vitro α- glucosidase inhibitory activity

In the evaluation of in-vitro α-glucosidase inhibitory activity, the ethanol extract of *Zappania urticifolia* (EEZU) demonstrated concentration-dependent inhibition. At 100µg/ml, EEZU exhibited a % α-glucosidase inhibition of 20.99±2.43, with an IC50 value of 352.39 µg/ml. Subsequent increases in concentration (200µg/ml, 400µg/ml, 800µg/ml, and 1000µg/ml) led to escalating inhibitory activities, reaching 46.26±1.26, 58.26±2.15, 68.21±1.98, and 88.35±1.65, respectively.

Table 1: Preliminary Phytochemical Analysis of various extracts of Z.urticifolia

Sl.No.	Test	P.ether	Benzene	Chloroform	Ethanol	Water
1.	Carbohydrates	-	-	-	+	+
2.	Alkaloids	-	-	-	+	+
3.	Glycosides	-	-	-	+	+
4.	Tannins	-	-	-	+	+
5.	Steroids	+	+	+	-	-
6.	Triterpenoids	+	+	+	+	-
7.	Volatile oils	-	-	-	-	-
8.	Fats and fixed oils	-	-	-	-	-
9.	Flavanoids	-	-	-	+	+
10.	Polyphenols	-	-	-	+	+
11.	Saponins	-	-	-	+	+
12.	Aminoacids	-	-	-	+	+
13.	Gums and mucilages	-	-	-	-	+

Table 2: Content of chemical constituents in the various extract of Zappania urticifolia

Extract	Percentage yield (%w/w)	Total Phenol Content
Pet ether	5.38	31.22±2.38
Benzene	2.56	22.28±2.18

Chloroform	10.25	18.05±3.42
Ethanol	26.64	255.67±19.56
Water	20.18	183.93±12.17

Table 3: In-vitro α-amylase inhibitory activity of Extract EEZU

Test sample	Concentration µg/ml	% α-amylase Inhibition	IC_{50} (µg/ml)
EEZU	100µg/ml	8.11±1.76	268.89 µg/ml
	200µg/ml	26.65±3.16	
	400µg/ml	43.65±1.32	
	800µg/ml	62.18±2.71	
	1000µg/ml	77.43±1.52	
Standard (Acarbose)	5µg/ml	14.23±1.44	28.48µg/ml
	10µg/ml	28.45±1.44	
	20µg/ml	48.71±0.68	
	40µg/ml	75.32±1.70	
	50µg/ml	88.81±1.85	

All values are mean ± SEM of 3 parallel measurements.

Table 4: In vitro α-glucosidase inhibitory activity of extract EEZU

Test sample	Concentration µg/ml	% α-glucosidase Inhibition	IC_{50} (µg/ml)
EEZU	100µg/ml	20.99±2.43	352.39 µg/ml
	200µg/ml	46.26±1.26	
	400µg/ml	58.26±2.15	
	800µg/ml	68.21±1.98	
	1000µg/ml	88.35±1.65	
Standard (Acarbose)	5µg/ml	12.12±2.15	26.08 µg/ml
	10µg/ml	31.54±1.79	
	20µg/ml	51.27±2.19	
	40µg/ml	71.19±2.11	
	50µg/ml	87.19±1.72	

All values are mean ± SEM of 3 parallel measurements.

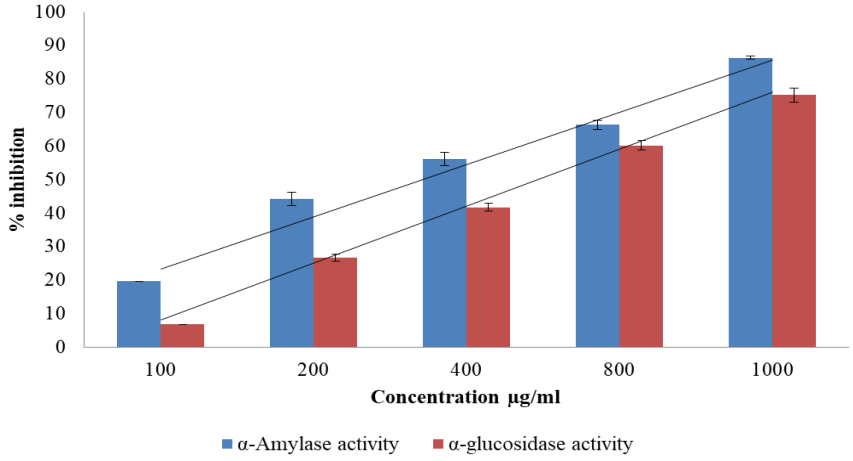

Figure 1.1: Invitro anti-diabetic activity of Zappania urticifolia (EEZU).

DISCUSSION

Zappania urticifolia, a plant known for its medicinal properties, has been the focus of this study to explore the potential therapeutic benefits of its ethanol extract (EEZU). In the broader context of natural remedies, understanding the phytochemical composition and pharmacological activities of such plant extracts becomes crucial. This study aimed to contribute valuable insights into the antioxidant, anti-lipid peroxidation, and anti-diabetic properties of EEZU, aligning with the global interest in plant-based medicine.

The phytochemical analysis of EEZU revealed a diverse range of chemical constituents, among which quercetin stood out prominently, as confirmed by High-Performance Thin-Layer Chromatography (HPTLC).The quantification of quercetin in EEZU not only adds to the understanding of its chemical composition but also provides a basis for the observed antioxidant effects.

EEZU demonstrated concentration-dependent inhibition of α-amylase, an enzyme involved in carbohydrate digestion. The IC50 value provides insights into the concentration required for 50% inhibition, with higher concentrations showing increased inhibitory effects.The comparison with the standard drug Acarbose emphasizes EEZU's effectiveness at higher concentrations, suggesting its potential as a natural anti-diabetic agent (Bailey, L.H. et al., 1941). investigated α-amylase inhibitory activities in various plant extracts, reinforcing our results on EEZU's concentration-dependent α-amylase inhibition. The α-glucosidase inhibitory activity observed in EEZU resonates with the broader understanding of plant-based anti-diabetic agents.

CONCLUSION

In conclusion, our study of Zappania urticifolia's ethanol extract (EEZU) has unraveled a captivating narrative of botanical potential. As we navigated the intricate pathways of phytochemistry, and a cascade of pharmacological assays, the story that unfolded was one of promise and possibility. In this conclusion, Zappania urticifolia stands not only as a subject of study but as a beacon in the vast landscape of botanical potential. As we applaud the symphony of results, the echo reverberates, not just within these laboratory walls but across the broader canvas of scientific inquiry. The journey may be concluding, but the story of Zappania urticifolia's ethanol extract is destined for sequels that promise to captivate and inspire future generations of researchers and seekers of natural remedies.

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