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Research Article

GC-MS ANALYSIS, *IN-VITRO* ANTI-DIABETIC AND ANTI-OXIDANT ACTIVITIES OF *Rhynchosia nummularia* (L.) DC

Sekar Vinoth Kumar *[1], Venkatachalam Gopal [2] and Nayakanti Devanna [3]
[1]Research scholar, Jawaharlal Nehru Technological University, Ananthapuramu, Andhra Pradesh, India
[2]Professor, HOD & Principal, Mother Theresa post graduate ad Research Institute of Health Sciences, Puducherry, India
[3]Director, Oil Technological and Pharmaceutical Research Institute, Ananthapuramu, Andhra Pradesh, India
*Corresponding Author Email: vinogkcp@gmail.com

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ABSTRACT

Rhynchosia nummularia (L.) DC has been used in the traditional medicine for the treatment of pain, inflammation and diabetes. There is no proper evidence available to justify its medicinal uses for this plant. The aim of the present study is to identify the active components by GC-MS and to screen the *in-vitro* anti-oxidant & anti-diabetic potential of *Rhynchosia nummularia*. The aerial part of the plant was extracted using ethanol. The active compounds present in ethanol extract of aerial parts of *Rhynchosia nummularia* (EERN) was identified through GC-MS analysis. The *in-vitro* anti-oxidant activity was determined by DPPH and ABTS method. The *in-vitro* anti diabetic activity was determined by α-glucosidase and α-amylase inhibitory activities. From the GC-MS analysis twenty one compounds were identified from EERN. The calculated IC_{50} of DPPH activity was 38.07 µg/ml, and for ABTS activity 19.8 µg/ml. EERN showed significant inhibition in both α-glucosidase and α-amylase. Hence, flavonoids and phenolic compounds present in the EERN responsible for antioxidant and anti-diabetic activities. From these results it was clearly evident that *Rhynchosia nummularia* has potent antioxidant and anti-diabetic activities.

Keywords: *Rhynchosia nummularia*, anti-diabetic, anti-oxidant DPPH and ABTS.

INTRODUCTION

Plants based drugs have been used in the treatment diseases for hundreds of years in Indian system of traditional medicine[1]. Majority of the people throughout worldwide still rely on the traditional system of medicine for their basic health care needs. As per WHO still 80% of the people from the developing countries uses plant based drugs as their primary choice of treatment[2]. World's population in developing countries like India is increasing at an alarming rate, which causes a huge demand in supply of medicines to peoples to satisfy their primary health care needs. Plant based drugs used in the traditional system of medicine has gained a huge attention because of their easy availability, low cost and lesser side effects. In India Ayurveda, Siddha and Unani system of medicines which primarily uses medicinal plants for the treatment of diseases for both human and veterinary use[3]. The genus *Rhynchosia* of twining or erect herbs found in the tropics of both hemispheres. About 22 species have been recorded in India; *Rhynchosia nummularia* (L.) DC used for anti-nociceptive, anti-inflammatory, anti-diabetic and anti-pyretic effects[4]. Hence the present work was taken to standardize and to identify the phyto-constituents present in the ethanolic extract of aerial parts of *Rhynchosia nummularia* (L.) DC (EERN) by GC-MS and to perform *in-vitro* anti-oxidant and anti-diabetic activities.

MATERIALS AND METHODS

Plant collection

The aerial parts of *Rhynchosia nummularia* were collected from coastal areas of Tirunelveli, in the month of November 2015 and were identified and authenticated by Fr. S.John Britto, Director, The Rapinat Herbarium and Centre for Molecular Systematics, St. Josephs College, Tiruchirapalli, Tamilnadu, A specimen of the plant material were preserved in the Department of Pharmacognosy, Swamy Vivekananda College of Pharmacy, Tiruchengode, Namakkal (Voucher specimen no: SVCP/Ph.Cog/85).

Extraction

The coarsely powdered plant was extracted with ethanol to obtain the crude ethanol extract of *Rhynchosia nummularia* (EERN). The ethanol extract was concentrated in a rotary vacuum evaporator yielding a semisolid residue. The extract was preserved in refrigerator at 4ºC for further analysis.

Preliminary Phytochemical screening

The EERN was subjected to qualitative chemical tests to identify various bioactive chemical constituents present in the plant using standard procedures for the presence of phenols, terpenoids, alkaloids, carbohydrates, tannins, flavonoids, and saponin[5, 6].

GC-MS analysis and compounds identification

The EERN were analyzed using a Perkin Elmer GC–MS equipment. The equipment consists of silica capillary column (30 m × 0.25 i.d) attached to MS. ESI method was used to deliver the samples into the mass spectroscopy. Helium was used as an inert gas at the flow rate of 1ml/min. EERN samples were dissolved in methanol HPLC grade and filtered using 0.2µm. The filtered EERN was injected with split mode with ratio of 1:120. The obtained spectrum was matched and identified with the known spectrum available in NIST library. The Chemical structure, molecular weight and chemical name of the identified compounds were listed and tabulated[7].

***In-vitro* antioxidant activity**

DPPH radical scavenging assay

DPPH solution 1ml was mixed with various concentrations of EERN and incubated in room temperature at dark for 30 min. The absorbance was measured at 517 nm. The DPPH radical scavenging activity was calculated using equation mentioned below[8]. The results were expressed in µg/ml

$$\text{Percentage of inhibition} = [(Abs_{Control} - Abs_{Sample}/\text{Standard})] / (Abs_{Control})] \times 100$$

ABTS radical scavenging assay

ABTS working solution were prepared from the stock solutions (7 mM ABTS and 2.4 mM potassium per sulphate). EERN at various concentrations were mixed with 1ml of ABTS working solution and incubate it for 7 min at room temperature and absorbance was recorded at 734 nm[9]. The ABTS free radical scavenging capacity of the extract was calculated from the following equation. The final result was expressed in µg/ml.

$$\text{Percentage of inhibition} = [(Abs_{Control} - Abs_{Sample}/\text{ standard})] / (Abs_{Control})] \times 100$$

***In-vitro* anti-diabetic activity**

α-glycosidase inhibitory activity

In a 96-well plate, reaction mixture containing 50 µl phosphate buffer (100 mM, pH = 6.8), 10 µl of α-glucosidase (1 U/ml), and 20 µl of varying concentrations of EERN was pre-incubated at 37°C for 15min. Then, 20 µl P-nitro-phenyl-α-D-glucopyranoside (5mM) was added as a substrate and incubated further at 37°C for 20 min. The reaction was stopped by adding 50 µl Na_2CO_3 (0.1 M). The absorbance of the released p-nitrophenol was measured at 405 nm using ELISA reader. Acarbose at various concentrations 50-300 µg/ml was included as a standard[10]. The results were expressed as percentage inhibition, which was calculated using the formula,

$$\text{Inhibitory activity (\%)} = (1 - As/Ac) \times 100$$

As is the absorbance in the presence of test substance and Ac is the absorbance of control

α-amylase inhibitory activity

Four concentrations of EERN were prepared by dissolving in deionized distilled water. In a 96-well plate, reaction mixture containing 50 µl phosphate buffer (100 mM, pH = 6.8), 10 µl α–amylase (2 U/ml), and 20 µl of varying concentrations of extract (50-300 µg/ml) was pre-incubated at 37°C for 20 min. Then, the 20 µl of 1% soluble starch (100 mM phosphate buffer pH 6.8) was added as a substrate and incubated further at 37°C for 30 min; 100 µl of the 3,5-dinitrosalicylic acid color reagent was then added and boiled for 10 min[11]. The absorbance of the resulting mixture was measured at 540 nm using ELISA reader (BioRad, USA). Acarbose at various concentrations (50-300 µg/ml) was used as a standard. The results were expressed as percentage inhibition, which was calculated using the formula,

$$\text{Inhibitory activity (\%)} = (1 - As/Ac) \times 100$$

As is the absorbance in the presence of test substance and Ac is the absorbance of control.

RESULTS

The results of the preliminary phytochemical analysis confirmed the presence of phenols, flavonoids, tannins, alkaloids and carbohydrates in EERN (Table 1). The results of the GC-MS analysis of EERN was illustrated in figure 1. Twenty one active compounds were identified from EERN. The identified components were tabulated as per the retention time. The molecular weight and the chemical formula of the identified compounds were compared with the data's available with NIST library (Table 2). Out of the twenty one compounds two compounds trehalose and (α - D-Glucopyranoside, O-α – D glucopyranosyl) were having significant pharmacological properties as per the previous literatures.

In-vitro antioxidant activity of EERN was represented in table 3. EERN processes significant DPPH scavenging activity with increasing concentration of extracts. The IC_{50} concentration of DPPH method was found to be 38.07µg/ml. Similarly, EERN showed significant scavenging activity in ABTS method with increased was concentrations of the extracts. At 50µg/ml ABTS method showed the maximum protection of 99.11%. The IC_{50} value of EERN in ABTS method was found to be 19.8µg/ml. EERN have produced dose dependent inhibitory activity in both α-glucosidase and α-amylase. The maximal inhibitory concentration (IC_{50}) values of α-glucosidase inhibitory activities of EERN at the maximum concentration at 300µg/ml have been 252.54 ± 0.28µg/ml correspondingly. The outcomes in Table 4 & Figure 2 showed that EERN exhibited strong activity almost in a dose-dependent way and is hence inferred to be an effective α-glucosidase inhibitor. EERN exhibited strong inhibitory activity against α-amylase at maximum concentration of 300µg/ml with 172.15 ± 0.93 µg/ml (Table 5 & Figure 3). Acarbose having α-glucosidase and α-amylase inhibitory activity of 134.76 ± 0.62 and 121.43 ± 0.61 µg/ml respectively.

Table 1: Preliminary phytochemical analysis of EERN

TEST	EERN
Carbohydrates	+
Proteins & amino acids	+
Glycosides	-
Alkaloids	+
Phytosterols	-
Flavonoids	+
Saponins	+
Tannins & phenolic compounds	+
Terpenoids	+
Gums	-

Table 2: GC-MS Spectral analysis of EERN

S. No.	RT min	Name of the compound	Molecular formula	Molecular weight
1	3.18	D-Glucose,6-O-alpha-D-Galactopyranosyl	$C_{12}H_{22}O_{11}$	842
2	3.80	DL-Arabinose	$C_5H_{10}O_5$	50
3	4.15	2-Acetamido-D-Mannitol	$C_8H_{17}NO_7$	239
4	4.50	2,4-Dihydroxy-2,5-Dimethyl,3(2H)-Furon-3-one	$C_6H_8O_4$	144
5	6.23	N-Methyl-3-Hydroxymethyl pyrrolidin-2-one	$C_6H_{11}NO_2$	129
6	7.21	5-Hydroxyuridine	$C_9H_{12}N_2O_7$	260
7	7.95	3,6-Dimethyl-5-Hepten-1-ol acetate	$C_{11}H_{20}O_2$	184
8	8.03	2,5-Dimethyl-4-Hydroxy-3(2H)-Furanone	$C_6H_8O_3$	128
9	8.74	Trehalose	$C_{12}H_{22}O_{11}$	343
10	9.12	N-(1-Methoxycarbonyl-1-methylethyl)-4-methyl-2-aza-1,3-dioxane	$C_7H_{13}NO_4$	175
11	9.44	Acetic acid-3-methyl-6-oxo-hex-2-enyl ester	$C_{11}H_{16}O_4$	212
12	10.28	Benzofuran,2,3-dihydro	C_8H_8O	120
13	11.25	Cyclohexanone-2-isopropyl-2,5-dimethyl	$C_{11}H_{18}O$	166
14	11.87	4-hydroxy-2-methyl acetophenone	$C_9H_{10}O_2$	150
15	12.58	1,3,5-tri azaadamantane-7 ol	$C_7H_{13}N_3O$	155
16	13.24	1,1-dodecanediol,diacetate	$C_{16}H_{30}O_4$	286
17	14.66	2,6-pyridine-di carboxaldehyde	$C_6H_5NO_2$	135
18	15.29	3',5'-Dimethoxy acetophenone	$C_{10}H_{12}O_3$	180
19	16.33	Megastigmatrienone	$C_{13}H_{18}O$	190
20	17.45	α - D-Glucopyranoside, O-α – D glucopyranosyl	$C_{20}H_{38}O_{11}$	454
21	18.11	L-(+)-ascorbic acid 2, 6-dihexadecanoate	$C_{10}H_{10}FN_3$	191

Table 3: IC_{50} for in-vitro antioxidant assay of EERN

S. No	Method	IC_{50} Value
1	DPPH method	38.07µg/ml
2	ABTS method	19.8µg/ml

Table 4: α-glucosidase inhibitory activity of EERN

Concentration (µg/ml)	Percentage inhibition (%) of α-glucosidase by		IC_{50} (µg/ml)	
	EERN	Acarbose	EERN	Acarbose
50	11.30 ± 0.17	17.93 ± 0.16	252.54 ± 0.28	134.76 ± 0.62
100	15.94 ± 0.84	35.93 ± 0.54		
150	31.94 ± 0.93	55.93 ± 0.40		
200	35.31 ± 0.76	65.72 ± 0.57		
250	49.03 ± 0.36	81.94 ± 1.43		
300	57.02 ± 0.12	94.38 ± 0.84		

Table 5: α-amylase inhibitory activity of EERN

Concentration (µg/ml)	Percentage inhibition (%) of α-amylase by		IC_{50} (µg/ml)	
	EERN	Acarbose	EERN	Acarbose
50	13.43 ± 0.74	22.31± 0.26	172.15 ± 0.93 µg/ml	121.43 ± 0.61 µg/ml
100	30.59 ± 0.31	45.63±0.95		
150	42.13 ± 0.52	64.93±1.32		
200	61.35 ± 1.14	78.14±1.29		
250	70.43 ± 1.05	84.85±2.04		
300	87.78 ± 1.42	96.32±1.63		

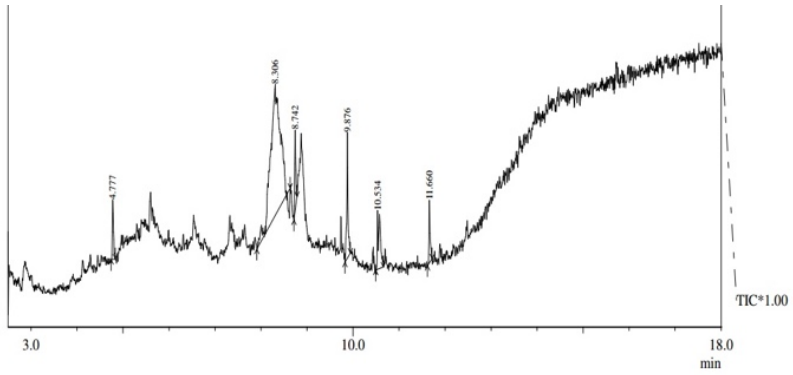

Figure 1: GC-MS chromatogram of EERN

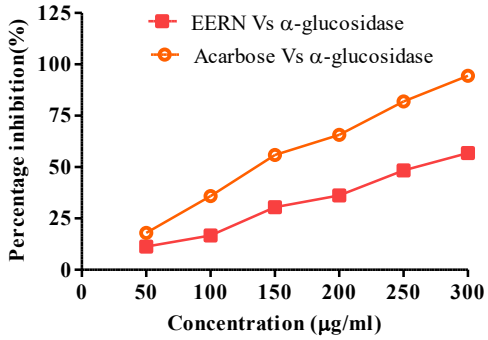

Figure 2: α-glucosidase inhibitory activity of EERN

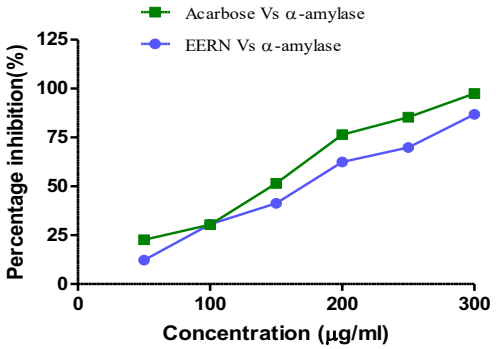

Figure 3: α-amylase inhibitory activity of EERN

DISCUSSION

Currently, many compounds derived from plant and marine sources were used as lead in the search of novel compounds in drug discovery. Identification of those novel leads can be primarily identified from phytochemical screening[12]. The results of the preliminary phytochemical analysis confirmed that various phytochemicals such as alkaloids, flavonoids, tannins and carbohydrate in the EERN. Recent interest in these substances has been stimulated by the potential health benefits arising from the antioxidant activities of these polyphenolic compounds[13]. Most recent researches have focused that flavonoids have anticancer, anti-inflammatory, antioxidant and anti-fungal activities and tannins have general anti-oxidant and antimicrobial activities[14]. Therefore, the compounds identified from *Rhynchosia nummularia* may be used a novel lead for treating many diseases.

GC-MS analysis is one of the important methods to predict the compounds present the plant extracts. It gives a broad idea about the metabolites present in the plant extracts. In the present study, two compounds with significant pharmacological properties were identified as per previous literatures. Previous reports showed that α - D-Glucopyranoside, O-α – D glucopyranosyl has protection against osteoporosis. DL-Arabinose, a monosaccharide used as commercial sweetener, is an inhibitor of sucrase, the enzyme that breaks down sucrose into glucose and fructose in the small intestine[15]. Plants enriched secondary metabolites such as, phenols and flavonoids exhibited superior antioxidant properties[16]. The EERN exhibit potent antioxidant property in both DPPH and ABTS methods. Many disorders like neuro degeneration, cancer and AIDS attributed through free radicals[17]. Generally, antioxidant tests using free radical traps relatively straightforward to perform. Among the free radical scavenging methods, DPPH is a sensitive and inexpensive method to analyze the plant extracts. When a DPPH solution mix with that substrate, it reduced the delocalization of electron with the loss of this violet color[18]. According to our study, the high phenolic content EERN can explain its high free radical scavenging activity.

Many bioactive compounds from different plants have been reported to have hypoglycemic effect, in that mostly phenols, triterpenoids and flavonoids have a positive correlation as anti-diabetic agents[19]. Carbohydrate rich diet causes rapid increase in the blood glucose level. The complex carbohydrate hydrolyzed rapidly with the aid of α-amylase and α-glucosidase enzymes and absorbed in the intestine as simple sugars (monosaccharides). Inhibitors of α-amylase and α-glucosidase have been useful as oral hypoglycemic drugs for the control of hyperglycemia especially in patients with Type II diabetes mellitus[20]. Both the inhibitors reduce blood glucose levels by reducing carbohydrate digestion and subsequently delay the glucose absorption. In the present study, EERN potentially inhibited both α-glucosidase and α-amylase enzymes. However, EERN at high concentration (300µg/ml), showed highest inhibition in both α-glucosidase and α-amylase. The plant based hypoglycemic agents with mild to moderate α-glucosidase and α-amylase inhibition will provide a successful treatment for diabetes by reducing the side effects caused by synthetic anti-diabetic drugs[21]. The inhibitory effect of EERN on both enzymes may be linked to the presence of saponins and alkaloids which may be acting individually or in synergy Thus, the result of the present study supports the traditional use of *Rhynchosia nummularia* in the treatment of diabetic and related diseases. However, further studies are needed on the isolation and detailed toxic and pharmacological aspects of EERN and their active components.

CONCLUSION

It can be concluded that, the ethanol extract of aerial parts of *Rhynchosia nummularia* has potential anti-oxidant, α-glucosidase and α-amylase inhibitory activity due to the phytochemical constituents present in the plant. However, further studies are required to isolate the bioactive components responsible for the activities.

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