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Original Article

PHYTOCHEMICAL SCREENING, ANTI-OXIDANT ACTIVITY AND IN VITRO ANTI-DIABETIC ACTIVITY OF AQUEOUS, METHANOLIC, ETHANOLIC AND CHLOROFORMIC EXTRACTS OF HYGROPHILAAURICULATA

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ABSTRACT

Objective: The objective of this study was to screen the phytochemical constituents of the seeds of the medicinal plant *Hygrophilaauriculata* and to obtain preliminary *in vitro* data about its anti-diabetic effects.

Methods: First extracts in four solvents i.e. water, methanol, ethanol and chloroform were prepared by optimized maceration procedures. Determination of the phytochemical constituents of these extracts was then carried out by employing standard procedures. Anti – oxidant activity was determined using the DPPH method. In order to determine the *in vitro* anti-diabetic activity, amylase inhibition studies and glucose diffusion inhibition studies were carried out.

Results: With the exception of the ethanolic extract, all the other extracts were found to be rich in phytochemicals. The methanolic extract contained the maximum number of phytochemicals. The antioxidant activity, alpha amylase inhibition and glucose diffusion inhibition were all found to be highest for the methanolic extract.

Conclusion: The present study proves that the methanolic extract of *H. auriculata* is a potent anti – oxidant and anti-diabetic. It can prove to be a valuable source of drugs targeting these diseases.

Keywords: Hygrophilaauriculata, DPPH, Anti-oxidant, Anti-diabetic, Phytochemical, Glucose Diffusion Inhibition, Ayurveda, Flavonoid, Methanolic Extract, Maceration.

INTRODUCTION

Hygrophilaauriculata is an herbaceous plant native to South India and parts of Africa. Its seeds are a part of South Indian cuisine, commonly used as a seasoning. It is known as *"Neermulli'* in the vernacular. It belongs to the Acanthaceae family and is one of the most versatile ayurveda medicines. All the different parts of the plant are prescribed for one or the other condition and it is very commonly prescribed by ayurveda doctors as an anti-diabetic agent. This holds great promise for a country like India where there are a huge number of diabetics. Due to the recent lifestyle changes and shift towards excessive urbanisation, there has been an exponential increase in the occurrence of diabetes. In countries with poor economies like India, it is useful to employ a number of indigenous plant medicines due to the relatively high cost of allopathic medicines [1]. Due to extensive folk medicine systems like ayurveda and siddha which have existed since prehistoric times, India is blessed with a wealth of ancestral knowledge about such plants. However, to ensure safety and efficacy, thecrude drugs derived from plants must be subjected to extensive phytochemical analysis to develop sustainable, safe and marketable drugs from them [2].

Apart from diabetes, lifestyle changes also cause an increase in the occurrence of other diseases like coronary artery diseases. It is a well – known fact that this disease occurs due to a higher fat intake in the diet. The current scenario where a large portion of the young Indian population is working and relies on fast food for nourishment, has led to a significant increase in coronary artery disease. Due to a busy schedule, these individuals are unable to take out time to exercise and thereby their chances of contracting coronary artery disease increase manifold. A relatively lesser known fact is that oxidative stress can cause an increase in the oxidation of low density lipoprotein and induce plaque formation in individuals [3]. A majority of plants that containeffective natural antioxidants like flavonoids can prevent and treatnearly all the oxidative related diseases [4].

The present study is aimed at investigating the potential of *H. auriculate* seeds as an antioxidant and an anti-diabetic agent. Though studies on this plant have been carried out earlier, no study has focused on the seeds of the plant [5]. With respect to India, it is of utmost importance that studies focusing on the seeds of the plant be carried out since it is the seed which is actually consumed as a food item by Indians.

MATERIALS AND METHODS

Plant Material

H. auriculate seeds were obtained from the local market in Vellore, Tamil Nadu, India.

Chemicals

The dialysis membrane, 1-4,α-D-Glucan-glucanohydrolase (α-amylase) andDPPH (2,2 diphenyl-1-picrylhydrazyl)were purchased from Hi Media Laboratories, Mumbai, India. All other chemicals and reagents used were of analytical grade and were procured locally.

Extract Preparation

The seeds were ground to a fine powder. The organic extracts were prepared by taking 50g of plant material with 250 ml solvent and placing on orbital shaker (The I L E Company, Chennai, Tamil Nadu, India) for 72 hours. The solvents were then filtered through muslin cloth and the filtrate was dried to yield extract. In case of aqueous extract, 50 g of the seed powder was mixed in 500 ml of water and put on the orbital shaker for 72 hours. This was done due to the formation of a gelatinous mass when smaller quantities of water were mixed with seed powder. The extracts were preserved at 4ºC till further use.

Phytochemical Screening

Phytochemical screening was performed using standard procedures to identify chemical constituents as described by Trease and Evans [6], Harborne[7] and Sofowora[8].

Anti-oxidant Activity by DPPH Method

Free radical scavenging activity of extracts was determined based on the DPPH spectrophotometric method of Mensor et al. with slight modifications [9].

1 ml of 0.3mM DPPH methanol solution was added to 2.5 ml solution of the extract (25, 50, 75 and 100 µg/ml) and allowed to react at room temperature for 30 minutes in the dark. The absorbance (Abs) of the resulting mixture was measured at 518 nm and converted to percentage antioxidant activity (AA %), using the formula:

$$AA\% = [100 - ((Abs_{sample} - Abs_{blank}) \times 100)]/Abs_{blank}$$

Methanol (1.0ml) plus extract solution (2.5 ml) was used as blank. 1 ml of 0.3mM DPPH plus methanol (2.5ml) was used as a negative control to set the spectrophotometer to zero. Ascorbic acid was used a positive control.

α – Amylase Inhibition

The percentage inhibition of the enzyme α – Amylase was determined. The procedure used was previously optimised and is detailed in Sathiavelu et al [10]. The only modification was the usage of Acarbose as a control.

Glucose Diffusion Inhibition

The glucose diffusion inhibition study was carried out as per the procedure detailed in Rastogi et al [11].

Statistical Analysis

All values are represented as Mean ± Standard Error Mean for a total of three values. All the results of the experiments were found to be significant values with a maximum tolerance level i.e. p – value of ≤ 0.05.

RESULTS AND DISCUSSIONS

Phytochemical Screening

The results of the phytochemical testing are depicted in Table 1. Out of the four extracts, the methanolic extract had the largest number of phytochemicals. Except anthraquinones, phlobatannins and cardenolides, the methanolic extract contained all the compounds. Since these compounds were not found in any of the four extracts, it is safe to assume that they are not present in the seeds of *H. auriculata*. The ethanolic extract had the least number of phytochemicals. Alkaloids were absent in the aqueous extract but present in all the other three. This can be attributed to the relative insolubility of alkaloids in water as compared to organic solvents[12],[13]. Cardiac glycosides and proteins were the only two phytochemicals present in all the extracts.

Table 1: It gives the results of the phytochemical screening of the extracts. A + sign indicates the presence of the compound and a – sign indicates the absence of it.

Test / Extract	Aqueous	Methanolic	Chloroformic	Ethanolic
Alkaloids	–	+	+	+
Flavonoids	+	+	+	–
Saponins	+	+	–	+
Phenols	–	+	–	–
Tannins	+	+	+	–
Anthraquinones	–	–	–	–
Cardiac Glycosides	+	+	+	+
Phlobatannins	–	–	–	–
Cardenolides	–	–	–	–
Terpenoids	–	+	–	–
Proteins	+	+	+	+

Anti – oxidant Activity

The anti – oxidant activity is listed in Table 2. The maximum antioxidant activity was displayed by the methanolic extract and the minimum was displayed by the ethanolic extract. This can easily be correlated with the results of the phytochemical screening. The methanolic extract was rich in flavonoids and tannins which are known to be potent anti – oxidants, especially flavonoids[14][15][16]. The chloroformic extract too, showed a significant anti – oxidant activity. However, this was lower than that of the methanolic extract. The aqueous extract showed a low anti – oxidant activity. However, it was higher than that of the ethanolic extract. These results could have direct implications for the mechanism of *H. auriculata's* anti-diabetic effect. A very interesting observation was that the anti – oxidant activity decreased with an increase in concentration of the extracts. We believe that this may be due to some solvation properties of the biomolecules, which may be affecting the bioavailability of the anti – oxidants. Alternately, due to an increase in concentration of the extract, some inhibitory substances that affect the activity of the anti – oxidants may also be increasing inadvertently. The methanolic extract showed an excellent anti – oxidant activity at all concentrations when compared to the extract. Despite its decreasing anti – oxidant with increasing concentration, the methanolic extract was a more potent anti – oxidant than ascorbic acid at all concentrations except the highest.

α – Amylase Inhibition

The results of the α – amylase inhibition study are given in Table 3. As is observable, the methanolic extract had the highest inhibitory action on the activity of the enzyme α – amylase. Here, the ethanolic extract and chloroformic extracts showed a nearly equal inhibitory action. The aqueous extract showed the least inhibition of α – amylase. Here, the inhibition increased with concentration, unlike in the previous experiment. From this, we concluded that the phytochemicals inhibiting the activity of the enzyme were not the same as those responsible for its anti – oxidant properties. Thus, they are most likely independent of the solvating properties that limit the bioavailability of other phytochemical classes. As compared to the standard acarbose, the methanolic extract had a much higher inhibitory effect. The other extracts, though comparable at lower concentrations, showed a much lower inhibitory effect at higher concentrations when compared to acarbose.

Glucose Diffusion Inhibition

The results of the glucose diffusion inhibition study are given in Table 4 and 5. While the results in Table 4 list the actual concentrations of glucose observed at different time intervals, Table 5 relates these concentrations to the control, thereby making it easier to understand their relevance. Here again, the methanolic extract showed the maximum inhibition to the movement of glucose outside the membrane. It managed to prevent the efflux of glucose forthe entire three hours. This could make it an extremely effective agent for controlling the post prandial blood glucose spike commonly experienced by diabetics. All other extracts showed fairly poor inhibition to glucose diffusion. Though the chloroformic extract managed to avoid the efflux of glucose, it could only do so for a short while.

Table 2: It shows the anti – oxidant activity of the extracts at four different concentrations as percentage free radical scavenging activity.

Conc(µg/ml)/Extract	Ascorbic Acid (%)	Aqueous (%)	Methanolic (%)	Ethanolic (%)	Chloroformic (%)
25	29.03±0.71	30.70±0.21*	74.69±0.15*	18.37±0.10*	52.64±0.11*
50	41.82±0.82	14.17±0.09*	68.52±0.08*	12.29±0.08*	45.13±0.21*
75	56.98±0.67	13.00±0.07*	60.05±0.11*	04.08±0.07*	28.62±0.12*
100	60.13±0.34	10.24±0.04*	53.22±0.23*	03.92±0.06*	16.30±0.09*

*Values are significant with p – value ≤ 0.05.

Table 3: It shows the percentage α – amylase inhibition of the extracts at four different concentrations.

Conc(µg/ml)/Extract	Acarbose (%)	Aqueous (%)	Methanolic (%)	Ethanolic (%)	Chloroformic (%)
25	34.62±0.06	17.06±0.08*	46.80±0.06*	22.62±0.04*	31.12±0.09*
50	41.71±0.03	19.25±0.05*	59.42±0.07*	29.21±0.09*	35.20±0.07*
75	55.39±0.12	24.47±0.09*	62.21±0.11*	34.14±0.07*	37.53±0.06*
100	64.98±0.09	29.52±0.10*	78.93±0.13*	46.35±0.14*	42.37±0.20*

*Values are significant with p – value ≤ 0.05.

Table 4: It shows the results of the glucose diffusion inhibition study at an extract concentration of 100 µg/ml in terms of concentration of glucose. The concentration of glucose is in mg/dl.

Time	Control	Aqueous	Methanolic	Ethanolic	Chloroformic
30	0.010±0.0003	0.009±0.0004*	0.003±0.0001*	0.088±0.0004*	0.006±0.0003*
60	0.134±0.0053	0.070±0.0035*	0.008±0.0016*	0.130±0.0013*	0.012±0.0001*
90	0.170±0.0077	0.128±0.0064*	0.010±0.0005*	0.149±0.0015*	0.042±0.0021*
120	0.198±0.0004	0.179±0.0039*	0.014±0.0003*	0.162±0.0081*	0.069±0.0022*
150	0.215±0.0107	0.185±0.0054*	0.021±0.0010*	0.182±0.0083*	0.197±0.0057*
180	0.243±0.0077	0.192±0.0080*	0.026±0.0090*	0.214±0.0077*	0.197±0.0098*

*Values are significant with p – value ≤ 0.05.

Table 5: It shows the results of the glucose diffusion inhibition study in terms of relative movement of glucose with respect to the control. The movement is expressed as a percentage.

Time	Control	Aqueous	Methanolic	Ethanolic	Chloroformic
30	100	90.00	30.00	80.00	60.00
60	100	52.24	05.97	97.01	08.96
90	100	75.29	05.88	87.65	24.71
120	100	90.40	07.07	81.82	34.85
150	100	86.05	09.77	84.65	91.63
180	100	79.01	10.70	88.06	81.07

CONCLUSION

Based on the results, we concluded that the methanolic extract of *H. auriculate* is both a potent anti – oxidant and anti-diabetic. This study provides scientific proof for the anti-diabetic and anti – oxidant properties of the *H. auriculata.* This plant can definitely be used for developing drugs targeting both diabetes and other oxidative stress related diseases. Before usage of the extract by humans is a possibility, clinical studies to test its safety and elucidate its mechanism of action are needed. To that end, we have planned animal studies in rats and have applied for ethical clearance of the same.

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CONFLICT OF INTEREST

The authors wish to declare that they have no conflict of interest.

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