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(RESEARCH ARTICLE)

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Study of antioxidant and anti-diarrheal activity of whole plant of Madhumalti {*Quisqualis indica* Linn.}

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Abstract

Hole plant of *Quisqualis indica* Linn. (Family Combretaceae) is commonly known as Rangoon creeper (combretum indicum) or madhumalati, traditionally used as anthelmintic The fresh plantwas studied for pharmaconostic evaluations, including examination of morphological and microscopic characters, determination of plantconstants, ash values and extractive values. The morphological studies revealed that the leaf is in dark green color with characteristic odour and slight bitter taste and. The shape of *Quisqualis indica* leaves is as ellipticalacuminate with entire margin, cordate base, and length varying from 7-12cm. Dorsal side is glabrous and ventral surface is hairy. Powder study revealed the presence of covering trichomes, annular xylem vessell, calcium oxalate crystals and anomocytic stomata. The stomatal index 18.75-19.02, vein islet number is 7-10, vein termination is 3-5, palisade ratio 6-7. The Moisture content, Total ash, acid insoluble ash, water-soluble ash values and sulfated ash were observed to be 8%, 9%, 12.5%, 6.55%and 5.45% w/w respectively. Water-soluble extractive values, Alcohol soluble extractive value and petroleum ether soluble extractive value of the leaves were observed to be 10%, 3% and1% w/w respectively. The phytochemical test revealed the presence of alkaloids, slight amount of glycosides, tannins, flavonoids and protein in both extract. Its flowers are used against diarrhea and eaten as vegetable. The flower extract gave high total polyphenol contents and showed strong antioxidant activity. In the search for new acetyl cholinesterase inhibitors from plant origin, it was demonstrated that methanolic extract of Q. indica flower exhibited this activity The extract inhibited electric eel acetyl cholinesterase in dose dependent manner with an IC_{50} value of 0.77 µg/m.

Keywords: *Quisqualis indica*; Pharmacognostic Evaluation; Phytochemical test; Anomocytic; Cordate

1. Introduction

In the last few decades there has been an exponential growth in the field of herbal medicine. It is getting popularize in developing and developed countries owing to its natural origin and lesser side effects. In olden times, *vaidyas* used to treat patients on individual basis, and prepared drugs according to the requirement of the patients. But the scene has been changed now; herbal medicines are being manufactured on a large scale in mechanical units, where manufacturers are facing many problems such as availability of good quality raw material, authentication of raw material, availability of standards, proper standardization methodology of drugs and formulations, quality control parameters and etc.1, 2 *Quisqualis indica* Linn (Combreteceae) is a strong climber, ligneous vine that can reach from 2.5 meters to up to 8 meters(Fig 1). It is commonly known as Rangoon creeper. It is indigenous in Africa, Indo Malaysian region and cultivated all over India3.Flower numerous, pendent, 7.5cm long, 3.8cm wide. At first they are white in colour then they become deep red. In amboynas, the leaves are given in a compound decoction for flatulent distension of the abdomen. In China the ripe seeds areroasted and given in diarrhea and fever. A popular anthelmenic among the inhabitants of North Annan4. There was no report on the extensive pharmacognostic studies of this plant species. Meanwhile, in this investigation the phytochemical studies of the leaves extract is also carried out. Tothe best of my knowledge, this is the first time the leaf was screened for pharmacognostic study.[1, 2, 3, 4,].

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2. Material and methods

2.1. Plant material authentication

The mature green leaves of *Quisqualis indica* Linn were collected in the morning locally from Jaipur District, Rajasthan, India, in the month of August 2009. The plant was identified and authenticated by the Botanist, from the Department of Botany, University of Rajasthan, Jaipur, India. A voucher specimen (RUBL20663) is deposited in the Department of Botany, University of Rajasthan.[2]

2.2. Pharmacogenetic studies

2.2.1. Macroscopy

Morphological studies were done by using simple microscope. The shape, apex, base, margin, taste and odor of leaves were determined.

2.2.2. Microscopy

Microscopic studies were done by preparing a thin hand section of midrib and lamina region of *Quisqualis indica* leaf. The section was cleared with chloral hydrate solution, stained with phloroglucinol and hydrochloric acid, and mounted with glycerin. (Fig 2) A separate section was prepared and stained with iodine solution for the identification of starch grains. Powder of the dried leaves was used for the observation of powder microscopical characters. The powdered drug was separately treated with phloroglucinol and HCl solution, glycerin and iodine solution to determine the presence of lignified cells, calcium oxalate crystals, trichomes and starch grains 5. [3, 4]

2.2.3. Quantitative microscopy

As a part of quantitative microscopy, stomatal number, stomatal index, vein islet and vein termination number were determined by using fresh leaves of the plant6. Total ash, water soluble ash, acid insoluble ash and sulphated ash were determined. Alcohol and water soluble extractive values were determined to find out the amount of water and alcohol soluble components.[7, 8]

2.2.4. Fluorescence analysis

Powdered leaf parts were subjected to analysis under day/visible light and ultra violet light after treatment with various chemical and organic reagents.9

2.2.5. Extraction and preparation of plant extract

The plant material leaves were air-dried at room temperature for 10 days and pulverized by grinder.Five hundred grams of the powered plant material was defatted with petroleum ether using cold maceration mehod for 7-8 days. The extract was then concentrated and greenish yellow semi solid mass (coded “PEE”) was obtained. The resulting marc was exhaustively extracted by same method using methanol and a greenish–brown mass coded “ME” was obtained after concentration on a water bath The extracts were stored aseptically in a desiccator at room temperature until demanded.

2.3. Phytochemical screening

The crude Petroleum ether extract and methanol extracts were screened phytochemically for thepresence of its constituents utilizing standard methods of analyses [10, 11, 12]

3. Results

The morphological studies revealed that the leaf is in dark green color with characteristic odour and slight bitter taste. (Table 1). The shape of *Quisqualis indica* leaves is as elliptical-acuminate with entire margin, cordate base, and length varying from 7-12cm. Dorsal side is glabrous and ventral surface is hairy(Table 2). Microscopic studies showed the presence of covering trichomes and glandular trichome. Midrib is having hypodermis which is made up of collenchymas. Lamina showed the presence of chlorenchyma next to epidermis. Midrib region showed xylem towards upper epidermis. Protoxylem found to move towards upper epidermis and meta xylem towards lower epidermal cells, Phloem moves towards lower epidermis. (Table 3) Powder study revealed the presence of covering trichomes, annular xylem vessel, epidermal cell, and anomocytic stomata.(Table 4). The fluorescence analysis of the powder drug was observed in day/visible light and UV light. (Table 5 and 6) The stomatal index 18.75-19.02, vein islet number is 7-10, vein

termination is 3-5, palisade ratio 6-7. (Table 7) The Moisture content, Total ash, acid insoluble ash, water-soluble ash values and sulfated ash were observed to be 8%, 9%, 12.5%, 6.55%and 5.45% w/w respectively.

Table 1 Identification of morphological feature

S.no	Features	Observation
01	Colour (Upper surface)	Dark green color
02	Colour (Lower surface)	Light green color
03	Odour	Characteristic
04	Taste	Tasteless
05	Shape	Ellipticle
06	Size	7-12cm
07	Arrangement	Opposite

Table 2 Botanical evaluation of *Quisqualis indica* linn. Leaf

S. No	leaf portion	observation
01	Apex	Acuminate
02	Margin	Entire
03	Shape	Ellipticle
04	Lamina	Pinnate
05	Venation	Reticulate
06	Midrib Contiuous	from base to apex
07	Dorsal surface	Glabrous
08	Ventral surface	Hairy
09	9 Petiole size	1 cm
10	10 Petiole shape	Cylindrical
11	Colour	Green
12	Leaf base	Cordate

Table 3 Transverse section of leaf

S.NO	Features	Observation
01	Trichomes	Present both glandular and covering
02	Upper epidermis	Present
03	Midrib	Hypodermis is made up of collenchymas
04	Lamina	After epidermis collenchyma is present
05	Midrib(vascular bundles)	Xylem towards upper epidermis. Proto xylem towards upper epidermis and meta xylem towards lower epidermal cells, Phloem towards lower epidermis.
06	Sclerenchyma	Present

Water-soluble extractive values, Alcohol soluble extractive value and petroleum ether soluble extractive value of the leaves were observed to be 10%, 3% and1% w/w respectively (Table 8). The qualitative chemical test revealed the presence of alkaloids, slight amount of glycosides, tannins, flavonoids and protein in both extract. Gum and mucilage is present in PEE and absent in ME. Carbohydrate is present in ME and slight present in PEE. (Table 9)[1, 5, 9, 10]

Table 4 Powder microscopy

S no.	Feature	Observation
01	Nature	Coarse powder
02	Colour	Light green
03	Odour	Charecteristic
04	Taste	Slight bitter
05	Covering trichome	Present
06	Xylem vessel	Present (Annular)
07	Epidermal cell	Present
08	Stomata	Present (Anomocytic)
09	Fibres	Present
10	Starch grain	Present
11	Calcium oxalate crystals	Present

Table 5 Analysis of powdered drug through naked eye

Reagents	Colour observed
Powder as such	Fade green
Powder + conc.HCL	Green
Powder + Conc.HNO3	Brown
Powder + Conc.H2SO4	Dark brown
Powder + Glacial acetic acid	Green
Powder + 5%NaOH	Brownish green
Powder + 5%KOH	Brownish green
Powder + 5%Ferric chloride	Dark green
Powder + Picric acid(saturated Aq. Solution)	Yellowish green
Powder + Ammonia	Brownish green

Table 6 Fluroscence analysis of powder drug

Chemical	Fluorescence Observed
Powder as such	Green
Powder + 1N NaOH in methanol	No fluroscence
Powder + 1N NaOH in water	Green
Powder + 50%HCL	Brown

Powder + 50%HNO3	Brown
Powder + 50%H2SO4	Green
Powder + Petroleum ether	Green
Powder + chloroform	Black
Powder + picric acid	Brown
Powder + 5%Ferric chloride solution	Green
Powder + 5% Iodine solution	Green
Powder + Methanol	Green
Powder + HNO3 + NH3	Green

Table 7 Data representing values of microscpical study

S. No	Microscopical parameter	Value
1.	Phloemfibre	Length:8.52-82.36, Width :1.09-1.42
2.	Calcium oxalate crystals:	Length:1.6-3.2, Width :1.4-1.6
3.	Starch grains	1.42-7.1
4.	Trichomes:	Length:15.62-5Width :1.42-2.84
5.	Stomatal no.	0.23-0.28
6.	Stomatal index	18.75-19.02
7.	Vein islet	7-10
8.	Vein termination no.	3-5
9.	Palisade ratio	6-7

Table 8 Data representing physiological parameter

01	Parameter	Values (%)w/w
	Loss on Drying	8% w/w
02	Ash values	
	Total Ash	9% w/w
	Acid insoluble ash	12.5% w/w
	Water soluble ash	6.55 w/w
	Sulphated ash	5.45%W/W
03	Extractive Values	
	Water soluble extractive	10% w/w
	Alcohol soluble extractive	3% w/w
	Petroleum ether soluble Extractive	1% w/w

Table 9 Phytochemical analysis of the petroleum ether and methanol extract of leaves of *Quisqualis indica* linn

S. No	Constituents/tests	Petroleum ether extract	Methanolic extract
Alkaloids			
1	Dragendorff's	++	-
2	Mayers	-	-
3	Wagners	++	++
4	Hagers	++	++
5	Tannic acid test	++	++
Glycosides			
1	Legal test	++	++
2	Baljet tet	-	-
3	Borntrager'test	-	-
4	Keller killiani test	+	+
Carbohydrates			
1	Molish test	+	+
2	Fehlings test	-	++
3	Barfoeds test	+	+
4	Test for starch	-	-
Protein and amino acid			
1	Biuret test	-	-
2	Xanthoproetic test	++	-
3	Copper sulphate test	-	++
Gum and mucilage			
1	Test with ruthenium red	-	++
Phytosterols			
1	Salkowski test	-	-
2	Libermanns burchard reaction-		-
Flavanoid			
1	Shinoda test	-	-
2	Lead acetate test	++	++
3	Ferric chloride test	-	++
4	Reaction with alkali and acid	+	++
Tannin and phenolic compound			
1	Lead acetate test	++	++
2	Ferric chloride test	++	++
3	Potassium dichromate test-	-	

Key: ++ = Highly present, + = faintly present, - = absent.

3.1. Assay of protein content of AChE

The protein content of AChE preparation was estimated by Bradford method using BSA (0-40 µg/ ml) as a standard (Bradford, 1976). All experiments were done in triplicate (n = 3). 22*Silpakorn U Science & Tech J Vol.1(2)*, *2007* Kinetics of Acetylcholinesterase Inhibition.[7, 8]

In vitro analysis of AChE activityIn order to select the proper concentration of enzyme, the AChE activity was measured in vitro byEllman method (Ellman et al., 1961), and each assay was done in triplicate (n = 3). The assay contained 1 ml of mixture of 0.25 mM ASCh and 0.25 mM DTNB in 50 mM sodium phosphate buffer pH 8 and 200 µL of AChE in different concentrations (0.01-0.243 µg/ ml). The final volume was adjusted to 3 ml with 50mM sodium phosphate buffer pH 8. The enzymatic reaction of AChE was the hydrolysis of acetyl group of ASCh and gave thiocholine (SCh) as the product. The SCh could react with DTNB to form 5- thionitrobenzoate, a colored anion, which absorbed UV at 412nm. The absorbances were measured at 0, 0.5 min and every 1 min interval starting from 0.5 min (0, 0.5, 1.5, …, 20.5). The rate of product formation (ΔA)was measured by the difference of absorbance (A) inevery 1 min time intervals within 20.5 min. Then theproduct formation was calculated for each AChEconcentration.[3, 4, 5]

3.2. The effect of plant extract on AChE substrate

HydrolyzationFor studying the effect of plant extract on AChEactivity, the enzyme was preincubated with each plantextract for 10 min before the addition of ASCh.

3.3. Estimation of the IC_{50} value

The concentration of the extract that inhibited 50% of AChE activity (IC_{50}) was estimated by method described by Kamal et al., 2000 and Alhomida et al., 2000. The method was performed by plotting % activity and %inhibition of AChE versus extract (inhibitor) concentrations on the same graph. The concentration at the intersection of these two curves was the IC_{50} value. The assay contained 200 µl of 0.0948 µg/ml AChE, chosen from *in vitro* analysis of AChE activity, 1 ml of mixture of 0.25 mM DTNB and 0.25 mM ASCh in 50mM sodium phosphate buffer pH 8 and 200 µl of plant extract in reaction concentration range of 0-2.22 µg/ml (final concentration). The final volume was adjusted to 3 ml with the 50mM sodium phosphate buffer pH 8.[2]

3.4. Estimation of kinetic parameters

Michaelis constants (Km) were determined by means of substrate concentration at 1/2Vmax of v and substrate concentration plot and Lineweaver-Burk plot over ASCh concentration range of 0.025-0.25mM (1/ASCh = 4-40 mM-1), while v and Vmax were velocity and maximum velocity, respectively. The assay contained 200 µl of 0.0948 µg/ml AChE, 1ml of mixture of 0.25 mM DTNB and different concentrations of ASCh (0.025-0.25 mM) in 50mM sodium phosphate buffer pH 8. The final volume was adjusted to 3 ml with the 50 mM sodium phosphate buffer pH 8. The assay conditions for measuring the inhibition activity of plant extract were the same as IC_{50} assay plus with 200 µl of plant extracts. The concentrations of plant extracts were in range 0 - 4.25 µg/3 ml of assay for each fixed concentration of ASCh. The kinetic values were applied by transforming [7]data of Lineweaver-Burk plot, Dixon plot, 1/Vmaxapp versus extract concentration plot and 1/Vmaxiapp versus 1/ASCh concentration plot. The Vmaxapp was the maximum apparent velocity of the AChE at the given concentration of extract (inhibitor). The Vmaxapp was obtained from the intersection at ordinate of Lineweaver-Burk plot. The Vmaxiapp was the maximum apparent velocity of the AChE in the presence of extract at the given concentration of ASCh and Vmaxiapp was obtained from the intersection at ordinate of Dixon plot. All graphs were plotted by Microsoft Excel. The correlation coefficient, slope, and intercept were obtained by linear regression and nonlinear regression analysis.[6]

4. Discussion

The data and plots (Figure 1b – 4) of *Q. indica*flower extract indicated the combination of the mixedand partially noncompetitive inhibition (Bisswanger, 2002). The mechanism of inhibition revealed that the extract might compete with ASCh for binding at substrate binding site of AChE or combined with AChE or with AChE-ASCh. In case of high concentration of ASCh, the extract may bind to the secondary binding site of AChE. This was confirmed by the increasing of Kmapp and decreasing of Vmax as the extract concentrations were increased. From KI value, they suggested that the extract had low affinity for AChE (Alhomida et al., 2000). The percentage inhibition of AChE of *Q. indica* flower extract increased upon the concentrations of extract. In previous report (Brossi, 1986), (-)-physostigmine salicylate showed inhibitory effect on electric eel AChE with IC_{50} value of 1.65 x 10-3 µg/ml. In this study, the flower extract of *Q.indica* exhibited inhibitory effect on that enzyme with an IC_{50} value of 0.77 µg/ml. So, the flower extract of *Q. indica* showed low AChE inhibitory activity when compared to the remark high potency physostigmine salicylate. Methanolic flower extract of *Q. indica* contained some active compounds that exhibited the AChE activity inhibition. However, this result

might be due to the synergistic effect of many compounds in this extract. Further purification and isolation should be performed for profoundly understanding the mechanism of AChE inhibitory activity. For this study, it is the first time screening the inhibition of acetylcholinesterase in *Q. indica* extract. So, the crude extract was used and further purification such as the elimination of tannin was not conducted. Somehow, some type of tannins might show the activity that should be further studied [11, 14, 15]

5. Conclusion

Acetylcholine is one of the most important neurotransmitter in either central or peripheral nervoussystem and the inhibition of AChE has been proposed as biomarker for the neurotoxicity. In this study, we have shown for the first time that *Q. indica* flower extract was dose-dependently inhibited the AChE activity in a noncompetitive manner. However, this extract showed slight inhibition of AChE activity (IC_{50} value of 0.77 µg/ml).

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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