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Phytochemical Analysis of Madhumalti Leaves (*Quisqualis indica* Leaves) Extract for Identification of Antioxidant and Other Immune Enhancer Constituents

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ABSTRACT

Rangoon Creeper scientifically known as Quisqualis indica belonging to family Combretaceae. Almost all of its parts are used to different ailments like antiflatulence, coughs; diarrhea, body pains, toothache, and cardiovascular system. The antioxidant potential of extract was evaluated using different antioxidant assays such as 1,1- -2-picrylhydrazyl (DPPH) assay, ferric reducing antioxidant using UV spectrophotometer. For the evaluation of delayed type of hypersensitivity (DTH) test animals were divided in to five groups, having six animals in each. Group I, the control, was given 2ml of 5% normal saline and to group II was vehicle control. III was standard drug. Group IV and V was administered of 200 mg and 100 mg/kg body weight of methanolic extract intraperitonially for ten days. The effect of test extract and standard drugs on the DTH response in Wistar rats using SRBCs as antigen, administration of methanolic extract of Q. indica at the dose of 100mg/Kg and 200mg/Kg and Levamisole 50mg/Kg treatments which were given orally. After 24, 48, 72, 96 hrs showed significant increase in paw edema compared to control group. So, this research suggested that these medicinal plants possess a significant antioxidant potential and are important source of natural antioxidants and can be effectively used in treating oxidative stress disorders.

KEYWORD: Quisqualis indica, Combretaceae, Antioxidant, Immune enhancer

1. INTRODUCTION

Free radicals such as hydroxyl, nitric oxide, hydrogen peroxide, superoxide anion, hypochlorite, lipid peroxides, and other singlet oxygen molecules are examples of reactive oxygen species (ROS) [1]. Our body naturally produces some substances known as free radicals throughout metabolic processes [2]. The body produces less free radicals during normal physiological activities; however, aberrant functioning or low antioxidant levels cause oxidative stress, which in turn causes the body to produce large amounts of these free radicals [3]. Numerous degenerative and chronic illnesses, including ischemic heart disease, Parkinson's disease, arthritis, cancer, stroke, Alzheimer's disease, immune suppression, atherosclerosis, ageing, diabetes mellitus, and chronic inflammatory diseases, are brought on by these free radicals [4]. However, in addition to natural causes, other variables like alcohol, smoking, ionising radiation, chronic disorders, and environmental pollution also contribute to the elevated levels of these free radicals [5]. Antioxidants, which are extremely important chemical substances, bind these free radicals and lessen and shield us from their damaging effects on the body's regular cells. While natural antioxidants are harmless and have few negative effects, some artificially created antioxidants, including butylated hydroxyl-toluene and butylated hydroxyl-anisole, are readily available on the market and are less stable. Antioxidants have historically been acquired from natural materials due to safety concerns [6]. To reduce the negative effects and damage caused by free radicals, natural food sources such as fruits, vegetables, seeds, herbs, sprouts, edible mushrooms, and cereals can be utilised as an effective source of antioxidants [7]. Phytochemicals are the chemical compounds found in plants that have protective properties. The primary phytoconstituents in plants—flavonoids, phenols, anthocyanins, iso-flavones, flavones, lignins, catechins, iso-catechins, and coumarins—are what give them their antioxidant potential. Total phenolic contents (TPC) and total flavonoid contents (TFC) are the primary methods used to measure these phytochemical constituents. The 1,1-diphenyl-2-picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP), and 2,2-azinobis-ethylbenzothiozoline-6-sulphonic acid (ABTS) assays are used to measure the antioxidant effects. [8, 9]

Therefore, employing a spectrophotometer to compare different medicinal plants using different procedures and evaluating free radical scavenging activity (RSA) are efficient methods for studying plants' secondary metabolites [10]. Quisqualis indica Linn

belongs to the Combretaceae family. Within the genus Quisqualis, Quisqualis indica Linn. is a particularly striking tropical vine that comes in a few different varieties that are identified by the size and colour of their leaves. Its leaves are oblong to elliptic, 7–15 cm long, with a rounded base and acuminate apex. They have a prominent venation. They are contrary and straightforward. In the tropics, it continuously and abundantly blooms throughout the year. [11, 12]

2. MATERIALS AND METHODS

2.1 Collection and Authentication of Plant: This study used four medicinal plants, which are believed to have important antilithiatic activities according to previous studies. The leaves of the *Quisqualis indica* were collected from Local region of Gwalior. The leaves were dried in shade. The leaves were authenticated by Department of Botany.

2.2 Chemicals: Unless otherwise stated, all chemicals used for the present study were of molecular biology grade, or spectroscopic grade. All chemicals were purchased from local suppliers, unless otherwise stated, such as Sigma-Aldrich, UK Ltd. (Gillingham, Dorset, UK), Fisher Scientific UK Ltd. (Loughborough, UK). All procedures were performed using the equipment available in the CDRI, Lucknow unless otherwise stated, such as FTIR, NMR, UV. Methanol, Ethanol, Hexane, Petroleum ether and Sulphuric acid were procured from S.D fine chemicals Pvt Ltd India.

2.3 Extraction of Drug: The leaves of *Quisquailis indica* should be air dried & make coarse powder with grinder. The coarsely powder leaves is packed in Soxhlet apparatus & continuously extracted with methanol at temp. at 60°-80°C till all the constituents is separated out. The success of the extraction with methanol is directly related to the extent that chlorophyll is removed into the solvent. When the tissue on repeated extraction is completely free of green colour, it can be assumed that all the low molecular weight compounds have been extracted. [13, 14]

2.4 Preliminary Phytochemical Investigation: Phytochemical tests were done in plant extracts for the detection of presence of different chemical constituents such as alkaloids, glycosides, flavonoids, essential oils, carbohydrates, proteins, tannins and other substances which are responsible for the biological activity. So, the chemical tests are performed in the methanolic extract of *Quisqualis indica* for the detection of different chemical constituents: [15]

2.5 In-Vitro Antioxidant Activity: In vitro antioxidant assays of the methanolic extract of *Quisqualis indica*. [16, 17]

2.5.1 DPPH Radical Scavenging Activity: The free radical scavenging activity of the extract was determined using. DPPH solution (0.004% w/v) was prepared in 95% methanol. The extract of *Quisqualis indica* was mixed separately with ethanol and to prepare the stock solution (5 mg/mL). Freshly prepared DPPH solution (0.004% w/v) was taken in test tubes and the extract was added followed by serial dilutions (1 µg to 500 µg) to every test tube so that the final volume was 3 mL and after 10 min, the absorbance was noted at 450 nm using a spectrophotometer. Ascorbic acid was used as a reference standard and was dissolved in distilled water to make the stock solution with the same concentration (5 mg/mL). Control sample was prepared containing the same volume without any extract and reference ascorbic acid. 95% methanol was served as blanks for extract. Percent scavenging of the DPPH free radical was measured by using the following equation:

% Scavenging Activity= [(Absorbance of the control – Absorbance of the test sample) / Absorbance of the control] X 100

2.5.2 Reducing Power: The reducing power of *Quisqualis indica* was performed accordingly as previously described. Different concentrations of the extract (200 to 1000 µg) in 1 ml of distilled water were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 ml, 1%). The mixture were incubated at 50°C for 20 min. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml. 0.1%) and the absorbance was measured at 450 nm. Increased absorbance of the reaction mixture indicated increased reducing power. Ascorbic acid was used as the standard. Phosphate buffer (pH 6.6) was used as blank solution. The absorbance of the final reaction mixture of two parallel experiments was taken and is expressed as mean ± standard deviation. [18, 19]

2.5.3 Pharmacological Investigation: 30 rates were divided in to five groups

- **Group I:** Control (normal saline)
- **Group II:** Vehicle Control
- **Group III:** Standard- Levamisole was administered at a dose of 50mg/kg/day by oral route for 14 days.

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- **Group IV:** *Q. indica* methanolic extract was administered at a dose 200 mg/kg/day by oral route
- **Group V:** *Q. indica* methanolic extract was administered at a dose 100 mg/kg/day by oral route

Delayed Type Hypersensitivity (DTH) Response: For the evaluation of delayed type of hypersensitivity (DTH) test animals were divided in to five groups, having six animals in each. Group I, the control, was given 2ml of 5% normal saline and to group II was vehicle control. III was standard drug. Group IV and V was administered of 200 mg and 100 mg/kg body weight of methanolic extract intraperitonially for ten days. On 10th day 0.1ml of SRBC solution was injected subcutaneously into the right footpad. After 24, 48, 72, 96 hrs, thickness of footpad was measured by plethisometer. Difference in the footpad thickness in control and treated group has been taken as the measure of the DTH reaction. [20-22]

3. RESULTS AND DISCUSSION

3.1 Extraction Of Drug

Percentage Yield of methanol extract *Quisqualis indica* is 9.45%.

Table 1: Percentage Yield of methanol extract

S. No.	Weight of Drug	% Yield
1.	100 gm	9.45

3.1.1 Characteristics of Extract

- Colour: Dark green
- Odour: Characteristics
- Taste: Characteristics

Figure 1: Extraction of Drug

3.2 PHYTOCHEMICAL STUDIES

Preliminary Phytochemical studies revealed the presence of phenolics compound & flavonoids were noticed in methanolic extract of *Quisqualis indica* leaves.

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Table 2: Data of the preliminarily phytochemical screening of *Quisqualis indica* methanolic leaves extract for alkaloids, glycosides and flavonoids

S. No.	Test performed	Results
1	Mayer's Test	Pr
2	Dragendroff's test	Pr
3	Wagner's test	Ab
4	Hager's test	Ab
5	Van-urk's for indole alkaloids	Ab
6	Vitali morin test for tropane alkaloids	Ab
7	Thalleoquin Test for Quinoline Alkaloids	Pr
8	Modified Born-Trager's Test	Ab
9	Test For Saponin Glycosides	Pr
10	Foam Test	Pr
11	Antimony Trichloride Test	Ab
12	Libermann Burchard Test	Pr
13	Raymond's Test	Ab
14	Kedde's Test	Pr
15	Baljet's Test	Ab
16	Xanthohydrate Test	Pr
17	Tollen's Test	Pr
18	Test for Coumarin Glycosides	Pr
19	Test for Cyanogenetic Glycosides	Pr
20	Test for hydroxyl anthrax quinines	Ab
21	Test for Cyanophoric Glycosides	Pr
22	Legal Test	Ab
23	Schonteten's Test	Ab
24	Bromine Test	Pr
25	Klunge's Isobar baloin Test	Ab
	Test for Flavonoid Glycosides	Pr

Pr= Present; Ab= Absent

Table 3: Preliminary phytochemical investigation for Tannin

S. No.	Test performed	Results
1	Goldbeater's skin test	Pr
2	Gelatin Test	Pr
3	Phenazone Test	Ab
4	Catechin test (matchstick test)	Ab
5	Chlorogenic acid test	Ab
6	Vanillin-Hydrochloric Acid Test	Pr
7	Gambir-flurescin Test	Pr

Table 4: Preliminary phytochemical investigation for Flavonoids

S. No.	Test performed	Results
1	Shinoda Test	Pr

Pr= Present; Ab= Absent

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Table 5: Preliminary phytochemical investigation for Carbohydrate

S. No.	Test performed	Results
1	Fehling's Solution Test	Pr
2	Molisch Test	Pr
3	Osazone formulation:	Ab
4	Resorcinol test for ketones (Selvinoff's test)	Ab
5	Test for pentoses	Pr
6	Killer-Kilani test for deoxy sugars	Ab
7	Furfural test	Pr
8	Benedict's Test	Pr

Pr= Present; Ab= Absent

Figure 2: Phytochemical investigation of methanolic extract

Methanol extract of the *Quisqualis indica* leaves shows the presence of carbohydrates, proteins, amino acids, steroids, phenolics compounds, flavonoids and saponin glycosides & absence of alkaloids, cardiac glycosides, anthraquinone glycosides and cynogenetic glycosides.

3.3 Antioxidant Activity:

DPPH (1, 1-diphenyl-2-picrylhydrazyl) Radical Scavenging Activity: The DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical scavenging activity of the extract of *Quisqualis indica* was given in Table 6. The IC_{50} values of the extract was found to be 0.15 µg/ml respectively whereas IC_{50} for ascorbic acid was found to be 0.12 µg/ml, which is a well-known antioxidant. *Quisqualis indica* methanolic extract showed moderate DPPH free radical scavenging effect as compared to standard antioxidants. DPPH is pink in solution and is a stable free radical, capable of accepting one electron from antioxidant containing plant extract and thus, neutralizing its free radical nature. The degree of decolourization indicates the scavenging activity of the plant extract and can be measured using UV spectrophotometer.

Table 6: DPPH scavenging activity (% inhibition) antioxidant activities of methanolic extract of *Quisqualis indica*

DPPH scavenging activity (% inhibition)		
Concentration in mg/ml	**Treatments**	
	Ascorbic acid	***Quisqualis indica***
0.2	1.65 ± 0.03	1.59 ± 0.01
0.4	1.78 ± 0.02	1.79 ± 0.02
0.6	2.43 ± 0.02	1.81 ± 0.01
0.8	2.67 ± 0.02	2.07 ± 0.02
1	2.72 ± 0.01	2.49 ± 0.02
EC_{50} (mg/ml)	0.12	0.15

Values are expressed as mean ± in standard deviation (N=6).

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Reducing Activity: The reducing power of the tested extract suggests low properties. Like other antioxidant assays, the reducing power of *Quisqualis indica* extract increased with increasing the amount of samples. The IC_{50} values of the extract was 0.12 µg/ml, whereas that for ascorbic acid was 0.009 µg/ml. The antioxidant activity of plant extract was accredited due to the presence of various antioxidants and their consequent mechanisms such as prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging, thus absence of one such antioxidant doesn't prevent an extract from showing strong antioxidant properties.

Table 7: Reducing power of antioxidant activity of methanolic extract of *Q. indica*

Absorbance at 450 nm		
Concentration in mg/ml	**Treatment**	
	Ascorbic acid	***Quisqualis indica***
0.2	1.72 ± 0.01	1.52 ± 0.01
0.4	1.86 ± 0.03	1.82 ± 0.03
0.6	2.41 ± 0.01	1.87 ± 0.02
0.8	2.78 ± 0.01	2.12 ± 0.01
1	2.81 ± 0.03	2.54 ± 0.01
EC_{50} (mg/ml)	0.09	0.12

Values are expressed as mean ± in standard deviation (N=6).

3.4 Determination Of Delayed Type Hypersensitivity Response: The effect of test extract and standard drugs on the DTH response in Wistar rats using SRBCs as antigen, administration of methanolic extract of *Q. indica* at the dose of 100mg/Kg and 200mg/Kg and Levamisole 50mg/Kg treatments which were given orally. After 24, 48, 72, 96 hrs showed significant increase in paw edema compared to control group.

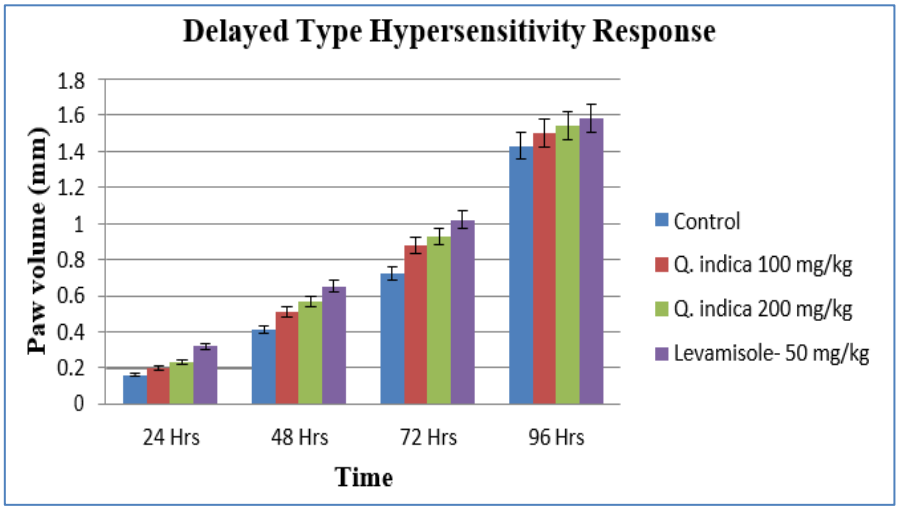

Figure 3: Show Delayed Type Hypersensitivity response

4. CONCLUSION

Q. indica, an important medicinal plant is one of the most widely cultivated species of the family Asteraceae. Various parts of the plant have been used for human medication. So that the present work was carried out with the objective of methanolic extract of leaves of Q. indica, phytochemical study, evaluation of immounomodulator activity. Many of the Quisqualis species have been reported for immunomodulatory activity. However, Q. indica leaves not been reported for the same. Phytochemical studies showed alkaloids, Phenolics, Flavonoids, carbohydrate, terpenoids, steroids, and saponins. Determined Delayed type hypersensitivity response show significance paw volume of lower and higher concentration as compared to control.

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Conflict of Interest Statement: All authors have nothing else to disclose.