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QUANTITATIVE ESTIMATION OF PHYTOCONSTITUENTS AND ANTI-HISTAMINIC ACTIVITY OF *QUISQUALIS INDICA* LINN

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Keywords:

Quisqualis indica, Folin-Coicalteu method, Aluminum chloride, Anti-cataleptic activity

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ABSTRACT: The objectives of the present study were to determine the total phenolic content, flavonoid content, and anti-cataleptic activity of chloroform and methanolic extract of the roots of *Quisqualis indica*. The crude extracts were investigated for total phenolic and flavonoid content using the Folin-Coicalteu method and the Aluminum chloride colorimetric method. It was found that the total phenolic content is greater than the flavonoid content in *Q. indica* roots. The total phenolic content of a methanolic extract is greater than the chloroform extract as 61.83 µg/ml and 25.40µg/ml, respectively. The flavonoid content in methanolic and chloroform extract was found to be 10.22µg/ml and 2.11µg/ml. The anti-cataleptic activity of the *Q. indica* roots extracts was evaluated by Wood block test. Catalepsy was induced by clonidine 1 mg/kg, subcutaneously and Chlorpheniramine maleate 10 mg/kg i.p as the standard drug. The vehicle-treated group has shown maximum duration of catalepsy (190.33±3.68) at 180 minute after the administration of clonidine. There was significant inhibition of clonidine induced catalepsy in the animal pre-treated with methanol and chloroform extract of *Q. indica* roots (400 mg/kg, p.o.). This suggests that the inhibition is through an antihistaminic action. Hence, we concluded that the methanol extract has more significant antihistaminic activity than the chloroform extract. The polar constituents in the methanol extract of *Q. indica* roots may be responsible for the antihistaminic activity and *Q. indica may* have a role in the treatment of asthma.

INTRODUCTION: Catalepsy is a condition where the animal maintains an imposed posture for long before regaining the normal posture. It is a sign of extra pyramidal effect of drugs that increases the histamine release in brain or inhibits the dopaminergic transmission. Catalepsy is induced by neuroleptic drugs, possibly due to a blockade of dopaminergic neurotransmission in the striatum.

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Neuroleptic-induced catalepsy is an animal model for screening drugs for Parkinsonism. The catalepsy test is usually used to evaluate drugs' motor effects on the extra pyramidal system. Evidence suggests that huge oxidative stress, free radical formation, genetic susceptibility and programmed cell death causes neuro-degeneration associated with Parkinson's and other associated diseases [1].

Quisqualis indica Linn (Combretaceae) is an evergreen plant growing all over India as an ornamental plant. The flowers are very attractive, showy white to pink colored with light fragrance. Fruits are dry, ovate-elliptic, 5-angled or 5-winged and seeded. The leaves are simple with acute apex and distinct venation [2, 3].

Seeds of the plant are used in folk medicine to treat helmenthiasis [4], roots decoction for rheumatism, coughs and tuberculosis; the fruits and seeds are used as anthelmintic, anti-emetic, anti-diarrhea [5]. The researchers have been scientifically investigated the folk claim of this plant. Few researchers have worked on the phytochemical investigation of the plant's leaves, flowers and stem. It possesses a number of pharmacological activities such as Anthelmintic [6], anti-pyretic [7], anti-inflammatory [8], insecticidal [9], immuno-modulatory [10], anti-diarrheal [11], anti-oxidants and anti-microbial [12] *etc*. due to the existence of various phytoconstituents. This plant's flowers and stem bark were evaluated for total tannins and anti-oxidant activity [13, 14]. There was no such report on roots. Henceforth, in the present study, we have investigated the phytochemicals, their quantification, and the anti-cataleptic activity of the *Q. indica* roots. The chloroform and methanolic extracts of *Q. indica* root (ChQIR and MQIR) were screened for total phenolic content and total flavonoid content, and anti-histaminic activity.

MATERİALS AND METHODS:

Collection and Authentication of Plant Material: The fresh roots of the plant *Quisqualis indica* Linn. were collected from healthy, fully grown plants from a residential area in Nashik, Maharashtra, India. The herbarium of the plant, including flowering top, stem and roots was prepared. One copy of herbarium was sent to the Botanical Survey of India (BSI), Pune, Maharashtra, India, for authentication and copy was restored in the department of Pharmacognosy, MVP's College of Pharmacy, Nashik. The plant material was assigned with authentication No. BSI/WRC/IDEN.CER/2016/403 (A).

Extract Preparation: The collected plant material was washed 2-3 times under the running tap water to get freed of dust particles and dried in direct sunlight at room temperature for about 3–4 weeks. The dried plant material was pulverized and sieved. The coarse powder was subjected to successive extraction by continuous hot percolation with petroleum ether (60-80°C) chloroform and methanol solvents. The liquid extracts were concentrated using a rotary vacuum evaporator (Evator) to get a semisolid extract.

Experimental Work:

Estimation of Total Phenolic Content: Total tannin content of both extracts *Q. indica* root were quantified by Folin-ciocalteu reagent following the method described by [15, 16]. The different concentrations of Gallic acid were used as a standard to plot the standard calibration curve. Both extracts were used in three different concentrations as 1000µg/ml, 750µg/ml and 500µg/ml. The Gallic acid used as a standard to plot the standard calibration curve. In 2 ml of each extract/standard concentration, 2 ml of previously diluted Folin-Ciocalteu reagent was added. Further, 2.5 ml of 20% sodium bicarbonate solution was added. The solutions were incubated in the dark for about 40min at room temperature. After the incubation, the absorbance of the blue color was measured at 725 nm spectrophotometrically (SHIMADZU, UV-1800). Total phenolic content was expressed based on a standard curve of Gallic acid, which was expressed as mg/g of gallic acid.

Estimation of Total Flavonoid Content: The flavonoid content of both ChQIR and MQIR extracts was estimated by Aluminium chloride methods [17, 18]. Quercetin was used as standard. The standard calibration curve was used to plot different concentrations as 10, 20,40,60,80 and 100µg/ml of Quercetin. The extracts concentrations as 1000µg/ml, 750µg/ml, and 500µg/ml were treated with reagents, and absorbance was measured at 415 nm on a spectrophotometer. The reaction mixture consists of 2 ml of extract /standard drug concentration, 0.1 ml of Aluminium chloride solution (10%) 0.1 ml of Potassium acetate solution, and 2.8 ml distilled water. This reaction mixture was incubated for 30 min, and after incubation, the absorbance of the reaction mixture was measured at 415 nm with a UV-visible spectrophotometer against a blank solution as methanol. The total flavonoid content of the extracts was calculated from the calibration curve.

Anti-inflammatory Activity by Carrageenan Induced Rat Paw Edema [19]: All the animals (Healthy Albino rats 15-200gm weight) were divided into ten groups, six animals in each group. All animals were treated with vehicle, diclofenac sodium, and plant extracts (200 and 400mg/kg, p.o.) one hour before Carrageenan injection.

0.1ml of 1% Carrageenan was injected into the sub plantar tissue of the right hind paw of each rat. Carrageenan-injected foot was swelled at 0, 1, 2, 3 and 4 h. using a digital vernier caliper immediately after carrageenan injection. The average foot swelling in drug-treated animal and standard was compared with that of control, and the percent inhibition (anti-inflammatory activity) of edema was determined using the formula.

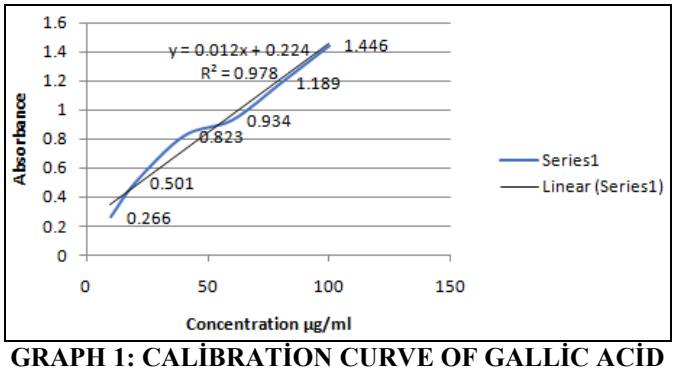
Percent inhibition = AB / A × 100

Where A represent edema volume of control and B as paw edema of test groups.

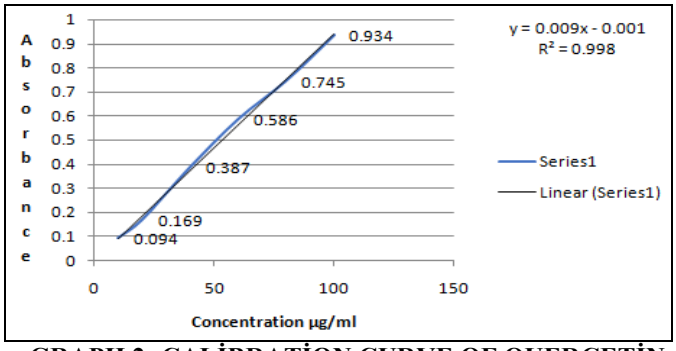
Anti-cataleptic Activity by Clonidine-Induced Catalepsy: The Wood bar test was preferred to study the effect of *Quisqualis indica* root extracts on Clonidine-induced catalepsy in mice [20, 21]. Mice were divided into six groups, six animals in each group. Group I (Control group) was given 1% Tween 80 solution 5ml/kg, orally; Group II (Standard group) was given a standard drug Chlorpheniramine male ate (10 mg/kg) intra peritoneally and Group III to Group VI were treated with the chloroform and methanolic extracts of *Quisqualis indica* roots ChQIR and MQIR each with two doses 200 mg/kg and 400 mg/kg respectively. All groups were given Clonidine 1mg/kg subcutaneously, 1 hour after the drug administration to induce catalepsy in mice. Both the forepaws of mice were placed on horizontal bar (1 cm in diameter, 3 cm above the table), and the time required to remove the paws from bar was noted for each animal, and the durations of catalepsy was measured at 30, 60, 90, 120and 180 min.

Statistical Analysis: The statistical analysis was performed by using one-way analysis-of-variance (ANOVA) followed by Dunnett's test for individual comparison of groups with control.

RESULTS: Total phenolic content was estimated by Folin-Ciocalteau's method. It is a colorimetric method based on the transfer of electrons between the reagents and polyphenols [22]. Phenolic compounds when treated phosphomolydbic acid and phosphotungstic acid present in the Folin-Ciocalteau reagent in an alkaline medium undergo redox reaction to produce blue colored complex. The intensity of blue color is directly proportional to the amount of tannin present in the sample. This method expresses the results as gallic acid equivalents mg/g of the extracts. The standard calibration curve of gallic acid and quercetin is shown in **Fig. 1** and **2**. The total phenolic content and total flavonoid content are given in **Table 1**. It was found that the total tannin content of the methanol extract (86.41mg/g) was greater than the chloroform extract (21.83mg/g). The total flavonoid content was found to be 3.22 and 17.77mg/g in ChQIR and MQIR, respectively.



GRAPH 1: CALİBRATİON CURVE OF GALLİC ACİD



GRAPH 2: CALİBRATİON CURVE OF QUERCETİN

TABLE 1: TOTAL PHENOLİC CONTENT AND TOTAL FLAVONOİD CONTENT OF THE *QUİSQUALİS İNDİCA* ROOT EXTRACTS

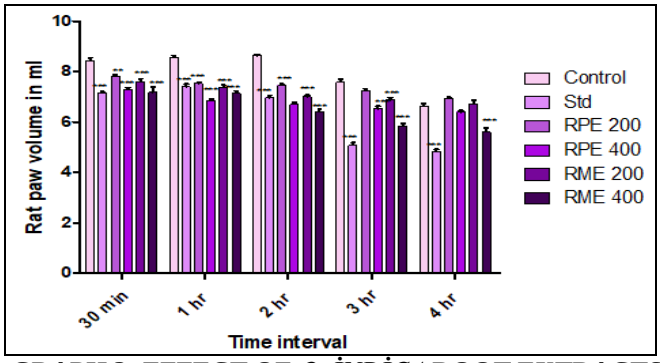
Plant Extract	Total Phenolic Content expressed as Gallic acid equivalents (mg/g of extract)	Total Flavonoid Content expressed as Quercetine quivalents (mg/g of extract)
Chloroform Extract	21.83	3.22
Methanolic extract	86.41	17.77

The anti-inflammatory activity of the extracts of *Quisqualis indica* was studied by using Carrageenan-induced rat paw edema in doses of 200 and 400 mg/kg. Both extracts showed

significant anti-inflammatory activity. The results are shown in **Table 2** and **Graph 3**. The results shown that the methanolic extract has shown the most significant action.

TABLE 2: THE ANTİ-İNFLAMMATORY ACTİVİTY OF *Q. İNDİCA* ROOT EXTRACTS ON CARRAGEENAN İNDUCED RAT PAW EDEMA

Group	Treatment	Paw volume in ml				
		30 min	1 hr	2 hr	3 hr	4 hr
Control	1% Tween 80 solution 5ml/kg Orally	8.44 ± 0.10	8.57 ± 0.06	8.62 ± 0.06	7.57 ± 0.12	6.62 ± 0.11
Standard	Diclofenac (10 mg/kg) Orally	7.15 ± 0.04	7.39 ± 0.11	6.95 ± 0.09	5.06 ± 0.11	4.82 ± 0.09
RPE 200	200mg/kg Orally	7.82 ± 0.04**	7.52 ± 0.05***	7.44 ± 0.06***	7.24 ± 0.07**	6.92 ± 0.07^{ns}
RPE 400	400mg/kg Orally	7.29 ± 0.07***	6.85 ± 0.05***	6.69 ± 0.09***	6.52 ± 0.11***	6.38 ± 0.06^{ns}
RME 200	200mg/kg Orally	7.60 ± 0.10***	7.38 ± 0.08***	7.00 ± 0.06***	6.87 ± 0.09***	6.72 ± 0.16^{ns}
RME 400	400mg/kg Orally	7.17 ± 0.22***	7.14 ± 0.09***	6.41 ± 0.10***	5.83 ± 0.11***	5.60 ± 0.16***



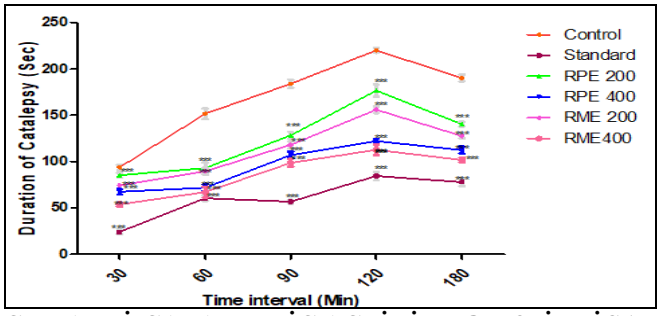
GRAPH 3: EFFECT OF *Q. İNDİCA* ROOT EXTRACTS ON CARRAGEENAN İNDUCED RAT PAW EDEMA

The anti-cataleptic activity of Petroleum ether (60-80^{O}C) and methanolic extracts of *Quisqualis indica* roots PEQIR and MQIR each with two doses 200 mg/kg and 400 mg/kg, respectively was determined by Clonidine induced catalepsy model in mice. The results are shown in **Table 3** and **Graph 4**. The graph is calculated by using Prism 5 software. From the results, it was found that both extracts showed significant anti-cataleptic activity. The 400 mg/kg methanolic extract has shown the most significant anti-cataleptic activity.

TABLE 3: ANTİ-CATALEPTİC ACTİVİTY OF PETROLEUM ETHER (60-80^{O}C) AND METHANOLİCEXTRACTS OF *Q. İNDİCA* ROOTS AGAİNST CLONİDİNE İNDUCED CATALEPSY MODEL

Groups	Conc. Mg/kg PO	Inhibition of Clonidine induced catalepsy				
		30 Min	60 Min	90 Min	120 Min	180 Min
Control (DW)	10ml/kg	93.66± 2.83	151.66± 5.73	184± 4.54	220.00± 2.49	190.33± 3.68
Standard	Chlorpheniramine maleate at 10 mg/kgi.p.	24.00± 2.16***	60.33± 3.09***	56.66± 1.96***	84.33± 4.64***	77.66± 4.18***
RPE	200	74.33± 1.24***	89.66± 3.74***	118.0± 3.85***	156.0± 3.67***	127.56± 2.49***
RPE	400	53.67± 2.05***	67.0± 5.79***	98.55± 4.08***	112.67± 5.71***	101.66± 2.48***
RCH	200	85.22± 2.49***	93.00± 6.01***	128.33± 3.74***	176.66± 6.48***	140.33± 2.49***
RCH	400	67.33± 2.94***	71.66± 3.74***	107.0± 3.66***	122.0± 2.49***	112.33± 3.68***

n=6; values are expressed in Mean±SEM. All groups compred with control group using Statistical analysis One Way ANOVA test followed by Dunnet's test.



GRAPH 4: CLONİDİNE İNDUCED ANTİ-CATALEPTİC ACTİVİTY OF *Q. İNDİCA* ROOTS EXTRACTS

DISCUSSION: The inflammatory process is a combination of various pathways like a synthesis of prostaglandin, interleukin, and platelet-activating factors. Inflammation initiates with any stress on the membrane or by other triggers, which activates hydrolysis of membrane phospholipid by phospholipase-A into arachidonic acid [23]. The inflammation induced by carrageenan is shown to be mediated by histamine and 5-HT during first 1 h, after which there is increased vascular permeability which is maintained by the release of kinins up to 1.30 h and from 1.30 to 3 h; the mediators appear to be prostaglandins. The release of prostaglandins is closely associated with migration of leucocytes into the inflamed site [24]. In this study the plant extracts significantly reduced the edema formation at 1, 2 and 3 h. The reduction in edema could be the action of extracts in the early phase, mainly by inhibition of the mediator of inflammation, probably by inhibiting the histamine and serotonin release from the pro-inflammatory cells like neutrophils and mast cells.

Catalepsy is a sign of the extra pyramidal effect of drugs that inhibit dopaminergic transmission or increase histamine release in the brain. Neuroleptic drugs are used to treat catalepsy. Cholinergic receptor antagonists have been used extensively to control the extra pyramidal side effects of neuroleptic drugs [25]. The catalepsy test is mostly preferred to evaluate motor effects of drugs acting on the extra pyramidal system. Evidence suggests enormous oxidative stress, free radical formation, genetic susceptibility, and programmed cell death that causes neuro-degeneration associated with Parkinson's and other associated diseases [26]. Clonidine, a α2-adrenoceptoragonist, induces dose-dependent catalepsy in mice, which histamine H1-receptor antagonists inhibit but not by H2-receptor antagonists [27].

CONCLUSION: The findings of this study specified that the plant *Q. indica* root possess antihistaminic activity that inhibited clonidine-induced catalepsy. From the present study, we can conclude that the cataleptic effect of clonidine in the mouse is mediated by histamine release from mast cells, and the clonidine-induced catalepsy was inhibited by chloroform and methanol extract of *Q. indica* root. The effect of these extracts on clonidine-induced catalepsy is probably due to their mast cell stabilizing properties. Methanolic extract of *Q. indica* root showed the most potent inhibition of clonidine-induced catalepsy compared to the chloroform extract. It is found that the total tannin content of *Q. indica* is greater than the total flavonoid content. So it can be concluded that polar constituents like tannins and flavonoids may be responsible for their antihistaminic activity.

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CONFLICTS OF INTEREST: None

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