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QUALITATIVE AND QUANTITATIVE ESTIMATION OF CHEMICAL CONSTITUENTS IN *NARINGI CRENULATA* (ROXB.) NICOLSON

***K. Sarada, P. Yokeswari Nithya and D. Shanmuga Priya**

Department of Chemistry, A.P.C. Mahalaxmi College for Women, Thoothukudi, Tamilnadu, India.

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***Correspondence for Author**
Dr. K. Sarada
Department of Chemistry, A.P.C. Mahalaxmi College for Women, Thoothukudi, Tamilnadu, India.

ABSTRACT

Naringi crenulata (Roxb.) Nicolson that belongs to Rutaceae family (subfamily- Aurantioideae) is a widespread species of the genus *"Naringi"*. It is commonly known as 'Mahavilvam' in Tamil. Preliminary chemical screening of petroleum ether (40^0- 60^0C), benzene, chloroform, methanol, ethanol and water extracts revealed the presence of chemical constituents such as alkaloids, coumarins, steroids, flavonoids, phenolic compounds, quinones, saponins, sugars, glycosides and anthraquinones. Quantitative estimation of alkaloids, phenolic compounds, saponins and flavonoids were carried out using uv-vis spectrophotometer and found that the ethanolic extracts contain the higher amount of steroids and flavonoids.

KEYWORDS: *Naringi crenulata*, phytochemical screening, uv-vis spectrophotometer.

INTRODUCTION

Plants are important sources of medicines and presently about 25% of pharmaceutical prescriptions in the United States contain atleast one plant derived ingredient. In the last century, roughly 121 pharmaceutical products were formulated based on traditional knowledge obtained from various sources. *Naringi crenulata* (Roxb.) Nicolson that belongs to Rutaceae family (subfamily- Aurantioideae) is a widespread species of the genus *"Naringi"*. It is commonly known as 'Mahavilvam' in Tamil. It has been used as folk medicine. The root extract is used for vomiting, dysentery and colic disorders. Fruit decoction is used as an antidote to insect poison. The bark juice is applied externally for getting speedy relief in sprain.[1] It is reported that its methanolic extract showed significant anthelmintic activity.[2] Pectic polysaccharides have been isolated from the fruits of *N. crenulata* by

extraction with water.[3] However, biological activities such as anticancer[4], hepatoprotectivity[5], aphrodisiac activity[6], anti-inflammatory activities[7] of ethanol extracts of leaf and bark of *N. crenulata* have also been reported in our earlier studies. The present study focuses on the chemical investigation of the chemical constituents of the different extracts of leaf and bark of *Naringi crenulata* responsible for these pharmacological activities.

MATERIALS AND METHODS

Collection and identification

The leaves were collected from well grown healthy plants inhabiting the natural forests of Agasthiarmalai Biosphere Reserve, Western Ghats, Tamil Nadu. The samples of *Naringi crenulata* were identified and authenticated by Prof. P. Jeyaraman, Plant Anatomy Research Centre, Chennai (PARC/2012/1382). The voucher specimen was deposited in the ethnopharmacological unit, Research Department of Botany, V.O.C. College, Tuticorin.

Preparation of extract

The leaf and bark of *Naringi crenulata* was dried in shade and homogenized to get a coarse powder. The powder was successively extracted with various solvents such as petroleum ether (40^0-60^0C), benzene, chloroform, methanol, ethanol and water.

Preliminary phytochemical screening

Standard procedures as suggested by Brindha[8] *et al*., Trease and Evans[9] and Harborne[10] were followed for preliminary phytochemical screening.

Quantitative Estimation of Alkaloids

To 1ml of ethanolic extract 5 ml pH 4.7 phosphate Buffer was added and 5 ml BCG solution and shake a mixture with 4 ml of chloroform. The extracts were collected in a 10-ml volumetric flask and then diluted to adjust volume with chloroform. The absorbance of the complex in chloroform was measured at 470 nm against blank prepared as above but without extract. Atropine is used as a standard material and compared the assay with Atropine equivalents.

Quantitative Estimation of Saponins

Ethanolic and aqueous extract was dissolved in 80% methanol, 2ml of Vanilin in ethanol was added, mixed well and the 2ml of 72% sulphuric acid solution was added, mixed well and heated on a water bath at 600c for 10min, absorbance was measured at 544nm against reagent blank. Diosgenin is used as a standard material.

Quantitative Estimation of Phenolic Compounds

The total phenolics content in different solvent extracts was determined with the Folin-Ciocalteu's reagent (FCR). Different concentrations of the extracts were mixed with 0.4 ml FCR (diluted 1:10 v/v). After 5 min 4 ml of sodium carbonate solution was added. The final volume of the tubes were made upto 10 ml with distilled water and allowed to stand for 90 min at room temperature. Absorbance of sample was measured against the blank at 750 nm using a spectrophotometer. A calibration curve was constructed using catechol solutions as standard and total phenolic content of the extract was expressed in terms of milligrams of catechol per gram of dry weight.

Quantitative Estimation of Steroids

1ml of ethanolic extract of steroid solution was transferred into 10 ml volumetric flasks. Sulphuric acid (4N, 2ml) and iron (III) chloride (0.5% w/v, 2 ml), were added, followed by potassium hexacyanoferrate (III) solution (0.5% w/v, 0.5 ml). The mixture was heated in a water-bath maintained at 70 ± 2^{0}C for 30 minutes with occasional shaking and diluted to the mark with distilled water. The absorbance was measured at 780 nm against the reagent blank.

Quantitative Estimation of flavanoids

Total flavonoid content was determined by Aluminium chloride method using catechin as a standard. 1ml of test sample and 4 ml of water were added to a volumetric flask (10 ml volume). After 5 min 0.3 ml of 5% Sodium nitrite, 0.3 ml of 10% Aluminium chloride was added. After 6 min incubation at room temperature, 2 ml of 1 M Sodium hydroxide was added to the reaction mixture. Immediately the final volume was made up to 10 ml with distilled water. The absorbance of the reaction mixture was measured at 510 nm against a blank spectrophotometrically. Results were expressed as catechin equivalents (mg catechin/g dried extract).

RESULTS AND DISCUSSION

The Preliminary chemical screening of petroleum ether (40^0- 60^0C), benzene, chloroform, methanol, ethanol and water extracts were presented in the table 1. Quantitative estimation of major chemical constituents such as alkaloids, steroids, phenolic compounds, saponins and flavonoids of ethanolic extract and aqueous extract were presented in the table 2 and 3.

Table 1. Preliminary chemical screening of the various extracts of *Naringi crenulata.*

Test	Petroleum ether		Benzene		Chloroform		Methanol		Ethanol		Water	
	NCL	NCB	NCL	NCB	NCL	NCB	NCL	NCB	NCL	NCB	NCL	NCB
Alkaloids	-	-	-	-	-	-	+	+	+	+	+	+
Anthraquinones	-	-	-	-	-	-	-	-	-	-	-	-
Catechins	-	-	-	-	-	-	-	-	-	-	-	-
Coumarins	-	-	-	-	-	-	+	+	+	+	+	-
Flavonoids	-	-	-	-	-	-	+	+	+	+	+	-
Phenols	-	-	-	-	-	-	-	+	-	+	-	-
Quinones	-	+	+	+	+	+	-	+	+	+	-	-
Saponins	-	-	-	-	-	-	+	+	+	+	+	-
Steroids	+	+	+	+	+	+	+	+	+	+	-	-
Tannins	-	-	-	-	-	-	+	+	+	+	+	+
Terpenoids	+	+	+	-	+	+	+	+	+	+	-	+
Sugar	-	-	-	-	-	-	+	+	+	+	+	+
Glycosides	+	+	+	+	+	+	+	+	+	+	-	+
Xanthoprotein	-	-	-	-	-	-	+	+	+	+	-	-

Table 2: Quantitative estimation of chemical constituents in the leaf of *Naringi crenulata.*

S.No	Chemical constituent	Standard	Ethanolic extract	Aqueous extract
1	Alkaloids	Atropine	198.46 µg	181.65 µg
2	Saponin	Diosgenin	98.01 µg	101.56 µg
3	Phenolic compound	Catechol	108.12 µg	74.32 µg
4	Steroids	Cycloartenol	281.02 µg	192.96 µg
5	Flavanoids	Catechin	251.19 µg	105.23 µg

Table 3: Quantitative estimation of chemical constituents in the bark of *Naringi crenulata.*

S.No	Chemical constituent	Standard	Ethanolic extract	Aqueous extract
1	Alkaloids	Atropine	221.23 µg	191.55 µg
2	Saponin	Diosgenin	194.91 µg	163.26 µg
3	Phenolic compound	Catechol	88.92 µg	72.02 µg
4	Steroids	Cycloartenol	247.77 µg	161.05 µg
5	Flavanoids	Catechin	150.79 µg	85.93 µg

Preliminary chemical screening of petroleum ether (40^0- 60^0C), benzene, chloroform, ethanol and water extracts revealed the presence of chemical constituents such as alkaloids,

coumarins, steroids, flavonoids, phenolic compounds, quinones, saponins, sugars, glycosides and anthraquinones. Among different solvents used for extraction in a series, ethanolic extract and aqueous extract showed positive results for many numbers of chemical compounds. It contains Steroids, Saponins, Alkaloids, Carbohydrates, flavonoids and phenolic compounds. From the results of quantitative estimation of alkaloids, steroids, phenolic compounds, saponins and flavonoids it was found that the ethanolic extract of both leaf and bark contains the higher amount of alkaloids, steroids and flavonoids.

CONCLUSION

Qualitative and Quantitative estimations of chemical constituents in the leaf and bark of *Naringi crenulata* revealed the presence of chemical consituents such as alkaloids, steroids, phenolic compounds, saponins and flavonoids. Further detailed study is required to target the isolation of the pure compound.

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