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Phytochemical evaluation of leaf extracts of *Naringi crenulata* (roxb.) Nicolson

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Abstract: The use of traditional medicines holds a great promise as an easily available source as effective medicinal agents to cure a wide range of ailments among the people particularly in tropical developing countries like India. The present study investigates the qualitative and quantitative analysis of the major bioactive constituents of *N. crenulata* leaf extracts. The extractive values of aqueous, acetone and chloroform extracts were found to be 11.34, 4.24 and 6.06 respectively. Qualitative phytochemical analysis of these three solvent extracts confirm the presence of Alkaloids, Saponins, Flavonoids and Phenolic compounds in all the three extracts; however, these phytochemicals were more significant in aqueous extract. Quantitative analysis was carried out using TLC method by different solvent system. Amongst various solvent systems, Butanol: acetic acid: water (9: 0.9: 0.1 v/v/v) shows maximum resolution and number of spots produced at long UV (365 nm) and under iodine vapours. The TLC chromatograms constituted different coloured phytochemical compounds with different Rf values. It can be conveniently used to evaluate the quality of different area samples. This indicates that the leaves can be useful for treating different diseases because the therapeutic activity of a plant is due to the presence of particular class of compounds and thus can serve as potential sources of useful drugs in future.

Key words: Bioactive; phytochemical compounds; chromatograms; therapeutic activity and drugs

Introduction

There is considerable interest in the screening of plant and other natural product extracts in modern drug discovery programmes, since structurally novel chemotypes with potent and selective biological activity may be obtained (Cragg *et al.*, 1997). A consideration of biological activity in addition to the isolation and structure elucidation stages in a phytochemical investigation may add a great deal to the overall scientific significance of the work. Phytochemicals are bioactive compounds found in plants that work with nutrients and dietary fibre to protect against diseases. They are non-nutritive compounds (secondary metabolites) that contribute to flavour colour (Johns, 1996; Craig, 1999; Agbafor and Nwachukwu, 2011).

Globally, medicinal plants have been unique sources of medicines and constituted the most common human use of biodiversity (Hamilton, 2004; Hiremath and Taranath, 2010). Nearly 70% world population (mainly in the developing countries) rely entirely on such traditional medical therapies as their primary form of health care (Bewaji *et al.,* 1985). The use of drugs derived from plants has been utilized as a source of many potent and powerful drugs for thousands of years all over the world (Lewis and Elvin-Lewis, 1977). Even in modern times, plant-based systems continue to play an essential role in health care and in the recent past increasing research evidence is getting accumulated, which clearly indicate the positive role of plant extracts for health care (Shabnam Javed *et al.*, 2012).

Scientists first started extracting and isolating chemicals from plants in the 18th century (Das *et al.*, 2003), since then it is a growing inventory and that has to look into at herbs and their effects in terms of the active constituents they contain. Many studies have highlighted several pharmacological properties in medicinal plants and their isolated constituents including anti-oxidant, anti-diabetes, antibacterial, antiviral and anti-ulcer activities (Melendez and Capriles, 2006; Wong *et al.*, 2006). The effects are dependent on the chemical constituents present in the plant used. A substance found in medicinal plants, containing the healing property of plants (Ballas and Marcolina, 2006) is known as the active principle. It differs from plant to plant and examples of active principles include: anthraquinones, flavonoids, glycosides, saponins, tannins etc. Plants also contain other compounds such as morphine, atropine, codeine, steroids, lactones and volatile oils, which possess medical values for the treatment of different diseases. In recent years, these active principles have been extracted and used in different forms such as infusions, syrups, concoctions, decoctions, infused

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oils, essential oils, ointments and creams (Walters *et al*., 2000; Meena Sahu *et al*., 2012).

Since most plants have medicinal properties, it is of utmost importance that their efficacy and toxicity risks are evaluated (Olagunjua *et al*., 2009). The Knowledge of the chemical constituents of these plants is desirable because such information will be of value for the synthesis of complex chemical substances. Such phytochemical screening of various plants is reported by many workers (Siddiqui *et al*., 2009; Ashok Kumar., *et al*., 2010). Therefore, the objective of the present research work was to perform the phytochemical analysis of three different extracts of fresh leaves of *N. cernulata*.

Materials and Methods

Leaf collection and identification

The leaf specimens were collected in the month of August from Kumbakonam, Tamil Nudu, India and authenticated by Professor N. Raaman, Herbal Science Laboratory, centre for Advanced Studies in Botany, University of Madras, Chennai. After a thorough investigation leaves were checked for any pathological disorders and contamination of other plants and were washed with distilled water.

Preparation of extracts

The fresh leaves (300 grams) were grounded into paste and were extracted with water for 12 h at room temperature. This process was repeated successively with chloroform and acetone for 72 h at room temperature until the color of the extract becomes pale. The extracts obtained were filtered separately using Whatmann No. 1 filter paper. This was repeated for 2 to 3 times and similar extracts were pooled together and dried on water bath until the constant weight with dry mass was obtained for solvent extracts. The residual extracts were stored in refrigerator at 4°C in small and sterile glass bottles. Percent extractive values were calculated by the following formula.

$$\text{Percent Extracts} = \frac{\text{Weight of dried extract}}{\text{Weight of leaf material}} \times 100$$

Thin Layer chromatography (TLC)

Pre-coated silica gel 60 F_{254} plates (E. Merck, Germany) were used. The TLC plates were developed in a chamber containing different solvent systems as the mobile phase. The chromatographs were developed and dried at room temperature. The spots in developed TLC plates were detected by exposing the plates to iodine vapours (Harborne, 1998; Raaman, 2006), observed under visible and under UV lamps (254 and 365 nm wavelength) to visualize the spots produced. The R_f values of the coloured spots were recorded.

$$R_f = \frac{\text{Distance traveled by the compound}}{\text{Distance traveled by the solvent front}}$$

Preliminary phytochemical screening

The different qualitative chemical tests were performed for establishing the profile of the leaf extracts for its chemical composition. The following tests were performed to detect various phytoconstituents present in them.

Detection of alkaloids (Evans, 1997)

Solvent free extract (50 mg) was stirred with few mL of dilute hydrochloric acid and filtered. The filtrate was tested carefully with various alkaloidal reagents as follows:

A. Mayer's test (Evans, 1997)

To a few mL of filtrate, a drop or two of Mayer's reagent was added by the sides of the test tube. A white creamy precipitate indicated the test as positive.

Mayer's Reagent

Mercuric chloride (1.358 g) was dissolved in 60 mL of water and potassium chloride (5.0 g) was dissolved in 10 mL of water. The two solutions were mixed and made up to 100 mL with water.

B. Wagner's test (Wagner, 1993)

To a few mL of filtrate, few drops of Wagner's reagent were added by the side of the test tube. A reddish-brown precipitate confirmed the test as positive.

Wagner's reagent

Iodine (1.27 g) and potassium iodide (2 g) were dissolved in 5 mL of water and made up to 100 mL with distilled water.

C. Hager's test (Wagner *et al*., 1996)

To a few mL of the filtrate, 1 or 2 mL of Hager's reagent (saturated aqueous solution of picric acid) was added. A prominent yellow precipitate indicated the test as positive.

D. Dragendorff's test (Waldi *et al*., 1965)

To a few mL of filtrate, 1 or 2 mL of Dragendorff's reagent was added. A prominent yellow precipitate indicated the test as positive.

Dragendorff's reagent

Stock solution

Bismuth carbonate (5.2 g) and sodium iodide (4 g) were boiled for a few min with 50 mL glacial acetic acid. After 12 h, the precipitated sodium acetate crystals were filtered off using a sintered glass funnel. Clear, red-brown filtrate, 40 mL was mixed with 160 mL of ethyl acetate and 1 mL of water and stored in amber-coloured bottle.

Working solution

Ten mL of stock solution was mixed with 20 mL of acetic acid and made up to 100 mL with water.

Detection of carbohydrates and glycosides (Ramakrishnan *et al.*, 1994)
The extract (100 mg) was dissolved in 5 mL of water and filtered. The filtrate was subjected to the following tests:

A. Molish's test
To 2 mL of filtrate, two drops of alcoholic solution of α-naphthol were added, the mixture was shaken well and 1 mL of concentrated sulphuric acid was added slowly along the sides of the test tube and allowed to stand. A violet ring indicated the presence of carbohydrates.

B. Fehling's test
One mL of filtrate was boiled on water bath with 1 mL each of Fehling solutions I and II. A red precipitate indicated the presence of sugar.

Fehling's solution
Fehling's solution I: Copper sulphate (34.66 g) was dissolved in distilled water and made up to 500 mL with distilled water.

Fehling's solution II: Potassium sodium tartarate (173 g) and sodium hydroxide (50 g) was dissolved in water and made up to 500 mL.

C. Borntrager's test (Evans, 1997)
To 2 mL of filtrate hydrolysate, 3 mL of chloroform was added and shaken. Chloroform layer was separated and 10% ammonia solution was added to it. Pink color indicated the presence of glycosides.

D. Legal's test
Fifty mg of the extract was dissolved in pyridine; sodium nitroprusside solution was added and made alkaline using 10% sodium hydroxide. Presence of glycoside was indicated by pink color.

Detection of saponins by foam test (Kokate, 1999)
The extract (50 mg) was diluted with distilled water and made up to 20 mL. The suspension was shaken in a graduated cylinder for 15 min. A two-cm layer of foam indicated the presence of saponins.

Detection of phytosterols (Finar, 1986)
Libermann-Burchard's test
The extract (50 mg) was dissolved in 2 mL of acetic anhydride. To this, one or two drops of concentrated H_2SO_4 were added slowly along the sides of test tube. An array of color changes showed the presence of phytosterols.

Detection of fixed oils and fats (Kokate, 1999)
Spot test: A small quantity of extract was pressed between two filter papers. Oil stain on the paper indicated the presence of fixed oil.

Detection of phenolic compounds
Ferric chloride test (Mace, 1963)
The extract (50 mg) was dissolved in 5 mL of distilled water. To this, few drops of neutral 5% ferric chloride solution was added. A dark green colour indicated the presence of phenolic compounds.

Detection of flavonoids
Alkaline reagent test
An aqueous solution of the extract was treated with 10% ammonium hydroxide solution. Yellow fluorescence indicated the presence of flavonoids.

Results

Plants owe their therapeutically potential by the presence of secondary metabolites. phytochemical analysis of the extracts obtained with various solvents is utmost important relating to various diseases. The preliminary phyto–profiling for the leaves of *N. crenulata* were carried out. The extract values, colours and consistencies of the extracts were depicted in Table 2.1. Extractive values of aqueous, acetone and chloroform extracts of *N. crenulata* leaves were found to be 11.34%, 4.24% and 6.06% respectively.

Phytochemical screening of *N. crenulata* leaf extracts indicated the presence of different classes of secondary metabolites that are essential in herbal medicine. Among the phytochemicals obtained were alkaloids, glycosides, saponins, flavonoids and Phytosterols. Aqueous extract showed very lesser amount (+) for alkaloids, while negative results were obtained for phytosteriods, fixed oils and fats. Carbohydrates flavonoids, glycosides and saponin showed moderate degree of precipitation (++) and higher degree of precipitation (+++) was noted for phenonlic compounds (Table 2.2).

Phytochemical compounds present in the acetone extract showed lesser degree of precipitation (+) except alkaloids (-) (Table 2.3) whereas in chloroform extract saponin, flavonoid and phenolic compounds showed very lesser degree of precipitation (+) while alkaloids, phytoteroids and fixed oil and fats showed moderate degree of precipitation (++). However negative results were obtained for carbohydrate and glycosides (Table 2.4). The results also showed that acetone and chloroform extract contains flavonoids (+), saponins (+) and phenonlic compounds (+) with very lesser degree of precipitation while the same phytochemical compounds showed higher degree of precipitation (++/+++) in the aqueous extract.

Table 2.1: Preliminary Phyto-Profile for *N. crenulata* leaf extracts.

Different Solvents	Colour of extracts Value	Consistency	Yield (%age) Extractive
Water Extract	Brown	Sticky	11.34
Acetone Extract	Black	Dry	4.24
Chloroform Extract	Dark brown	Sticky	6.06

Table 2.2: Phytochemical screening of aqueous extract of *N. crenulata* leaves.

S. No	Test	Test applied/ Reagent used	Observation	Inference	Intensity
1	Alkaloids	Mayer's	No milky precipitate	Absent	-
		Wagner's	Reddish brown precipitate	Present	+
		Hager's	Yellow precipitate	Present	+
		Dragendroff's	Brick red precipitate	Present	+
2	Carbohydrate	Molish's	Violet ring formation	Present	+
		Fehiling's	Red precipitate	Present	++
3	Glycosides	Borntrager's	Pink color	Present	+
		Legal's	Pink color	Present	++
4	Saponins	Foam test	Foam formation	Present	++
5	Phytosterols	Libermann-Burchard's	No color changes	Absent	-
6	Fixed oil & Fats	Spot test	No oil stain on the paper	Absent	-
7	Phenolic compounds	Ferric chloride	Dark green color	Present	+++
8	Flavonoids	Alkaline reagent	Yellow fluorescence	Present	++

Table 2.3: Phytochemical screening of acetone extract of *N. crenulata* leaves.

S. No	Test	Test applied/ Reagent used	Observation	Inference	Intensity
1	Alkaloids	Mayer's	No milky precipitate	Absent	-
		Wagner's	No reddish-brown precipitate	Absent	+
		Hager's	No yellow precipitate	Absent	-
		Dragendroff's	No brick red precipitate	Absent	-
2	Carbohydrate	Molish's	Violet ring formation	Present	+
		Fehiling's	No red precipitate	Absent	-
3	Glycosides	Borntrager's	Pink color	Present	+
		Legal's	No pink color	Absent	-
4	Saponins	Foam test	Foam formation	Present	+
5	Phytosterols	Libermann-Burchard's	Color changes	Present	+
6	Fixed oil & Fats	Spot test	Oil stain on the paper	Present	+
7	Phenolic compounds	Ferric chloride	Dark green color	Present	+
8	Flavonoids	Alkaline reagent	Yellow fluorescence	Present	+

Table 2.4: Phytochemical screening of chloroform extract of *N. crenulata* leaves.

S. No	Test	Test applied/ Reagent used	Observation	Inference	Intensity
1	Alkaloids	Mayer's	No milky precipitate	Absent	-
		Wagner's	Reddish brown precipitate	Present	++
		Hager's	No yellow precipitate	Absent	-
		Dragendroff's	Brick red precipitate	Present	+
2	Carbohydrate	Molish's	No violet ring formation	Absent	-
		Fehiling's	No red precipitate	Absent	-
3	Glycosides	Borntrager's	No pink colour	Absent	-
		Legal's	No pink colour	Absent	-
4	Saponins	Foam test	Foam formation	Absent	+
5	Phytosterols	Libermann-Burchard's	Color changes	Present	++
6	Fixed oil & Fats	Spot test	Oil stain on the paper	Present	++
7	Phenolic compounds	Ferric chloride	Dark green color	Present	+
8	Flavonoids	Alkaline reagent	No yellow fluorescence	Absent	+

Thin layer chromatographic technique is a useful analytical tool for the isolation and identification of organic compounds. The data of quantitative separation of secondary metabolites from *N. crenulata* leaf extracts by thin layer chromatography are summarized in Table 2.5. Rf values obtained by thin layer chromatography patterns are useful to establish their identity and purity of the medicinal plants. The plates were first exposed to visible light, then viewed through UV (254 & 365 nm), and kept in iodine chamber to observe the variously coloured bands. The TLC pattern is displayed in Figure 2.1. The Rf values and colour of each spot are tabulated (Table 2.5). The TLC profile of *N. crenulata* leaf extracts were established carried out by different solvent system**.** Amongst various solvent systems, butanol: acetic acid: water (9: 0.9: 0.1 v/v/v) shows maximum resolution. Number of spots produced at long UV (365 nm) and under iodine vapours.

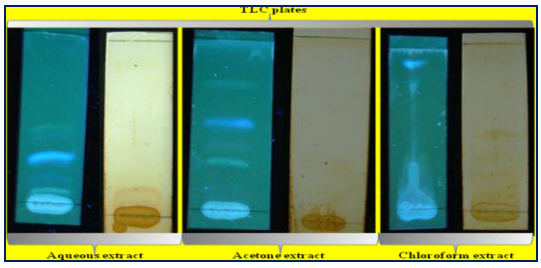
TLC chromatogram under visible light and short UV revealed no bands for all the three extracts. The aqueous, acetone and chloroform extracts under long UV revealed 5, 3 and 1 bands respectively and under iodine vapours, the three extracts revealed 2, 1 and 4 bands respectively. In all the three extracts the highest Rf value under long UV was 0.9 with Blue fluorescent band in chloroform extract and the least Rf value was 0.06 with Light-blue coloured band in aqueous extract. Similarly, in all the three extracts the highest Rf

value under iodine vapours was 0.9 with Yellow band in aqueous extract and the least Rf value (0.18) with Yellow coloured band was also observed in the same extract.

Table 2.5: TLC analysis of various extracts of *N. crenulata* leaves.

	Inference	Number of spots	Color of band	Rf values
Aqueous	Under visible light	0	-	-
	Under short U.V.	0	-	-
	Under long U.V.	5	Light-blue, Dark-blue, Blue fluorescent, Blue, yellowish	0.06, 0.1, 0.3, 0.56, 0.58
	Under Iodine vapour	2	Yellow	0.18, 0.9
Acetone	Under visible light	0	-	-
	Under short U.V.	0	-	-
	Under long U.V.	3	Light-blue, Blue fluorescent, Light-blue	0.38, 0.52, 0.74
	Under Iodine vapour	1	Yellow	0.34
Chloroform	Under visible light	0	-	-
	Under short U.V.	0	-	-
	Under long U.V.	1	Blue fluorescent	0.9
	Under Iodine vapour	4	Yellow	0.24, 0.34, 0.56, 0.92

Figure 2.1: TLC fingerprints of various extracts of *N. crenulata* leaves.



Discussion

Phytomedicine represents one of the most important fields of traditional medicine all over the world and are of prime importance to the health of individuals and communities. The medicinal values of these economically important plant species is due to presence of some chemical substances which produce a definite physiological action on human body like alkaloids, tannins, flavonoids and saponin etc. (Edeoga *et al.*, 2005; Khan *et al.*, 2011). To promote the proper use of phytomedicine and to determine their potential as sources for new drugs, it is essential to study medicinal plants, which have folklore reputation in a more intensified way (Subramanian and Suja, 2011). In the present study, the quantitative analysis of *N.* crenulata leaf extracts was carried out in fresh leaf samples. Alkaloids, flavonoids, saponins, glycosides, phytosterols and phenolic compounds were revealed to be present in *N.* crenulata leaf extracts. This shows high level of its possible medicinal values (Oloyed, 2005; Aja *et al.*, 2010; John *et al.*, 2011).

Screening of plants for medicinal value has been carried out by number of workers with the help of preliminary phytochemical analysis (Dan *et al.*, 1978; Ram, 2001; Mungole and Chaturvedi, 2011). Phytochemical screening is of paramount importance in identifying new source of therapeutically and industrially valuable compound having medicinal significance, to make the best and judicious use of available natural wealth. A number of medicinal plants have been chemically investigated by several workers (Battacharya *et al.*, 1971; Kokate *et al.*, 1998). The selection of plant part which yields maximum secondary metabolites is the prime or prerequisite step in this investigation. For this, different phytochemicals from *N. crenulata* leaf extracts were extracted by using water, acetone and chloroform solvents. Different types of solvent plays an important role in extractability of different phytochemical (Mungole and Chaturvedi, 2011).

Qualitative screening confirmed the presence of Alkaloids, Saponins, Flavonoids and Phenolic compounds in all the three extracts; however, these phytochemicals were more significant in aqueous extract. The present of these four compounds support the use of the plant in folklore medications. Alkaloids are known to contain a lot of pharmacological properties. They are mostly used as antidepressant (morphine), stimulants (caffine), anaesthetic (cocaine), antitumor (vinblastine) antimalaria (quinine), antibacterial (berberine) and amoebicide (emetine) (Bruneton, 1999; Cowan, 1999; Heinrich *et al.,* 2004*;* Gurib-Fakim, 2006). Saponins are glycosides possessed antimicrobial and inhibit Na^+ efflux, by blockage of the entrance of the Na^+ out of the cell, reducing congestive heart failure (Abou-Donia *et al.,* 2008). These compounds are known to be immune booster and are said to demonstrate anti-inflammatory, homolytic, allelopathic, cholesterol lowering and anticancer properties (Sauvaire *et al.,* 1996; Mandeau *et al.,* 2005). Flavonoids are known to have anti-inflammatory, anti-allergic, antiviral, antispasmodic and diuretic effect (Cowan, 1999). While Phenolic compounds have attracted a great attention in relation to their potential for beneficial effects on health. Over the last few years, several experimental studies have revealed biological and pharmacological properties of phenolics compounds, especially their anti-inflammatory

activity (Castillo *et al.*, 1989; Zhu *et al.*, 1997), antiviral, and cytotoxic activity (Chhabra *et al.*, 1984). It is a well-documented fact that most medicinal plants are enriched with phenolic compounds and bioflavonoids that have excellent antioxidant properties (Shirwaikar *et al.*, 2003; Mungole and Chaturvedi, 2011). Phenolics are active in curing kidney and stomach problems.

Thin layer chromatographic technique is a useful analytical tool for the isolation and identification of organic compounds. Rf values obtained by thin layer chromatography patterns are useful to establish their identity and purity of the medicinal plants. TLC method for the separation of phytochemicals were also reported in *Radix Polygoni* by Gao *et al.,* (2007) and in *Mucuna pruriens* by Misra and Wagner (2007) and in *Vitex trifolia* by Alfi *et al.,* (2010). In the present study, each solvent extract had different coloured bands. The aqueous, acetone and chloroform extracts under long UV revealed 5, 3 and 1 bands respectively and under iodine vapours, the three extracts revealed 2, 1 and 4 bands respectively. Gabriela (2009) suggested that the colours of the separated spots in TLC and their position relative to standard substances are important characteristics for the plant extract identification.

The present study showed the presence of important classes of phytoconstituents like alkaloids, carbohydrates, glycosides, Saponins, phenolic compounds and flavonoids in *N. crenulata* leaf extracts. This indicates that the leaves can be useful for treating different diseases because the therapeutic activity of a plant is due to the presence of particular class of compounds and thus can serve as potential sources of useful drugs in future.

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