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Research Article

PHYTOCHEMICAL SCREENING OF *NEOLAMARCKIA CADAMBA* AND ITS ROLE IN PREVENTING THE GROWTH OF BACTERIA ASSOCIATED WITH KIDNEY STONE

Prathibhakumari. P.V* and Prasad G

*Department of Zoology, Mar Ivanios College, University of Kerala, Thiruvananthapuram.

Department of Zoology, University of kerala, Karyavattom, Thiruvananthapuram, Kerala.

ABSTRACT

The present study was carried out with an objective to determine the antibacterial property of *N. cadamba* fruit extract (low and high concentration) against gram positive (*Staphylococcus aureus*) and gram negative (*Escherichia coli, Pseudomonas aeruginosa, Proteus mirabilis* and *Klebsiella pneumonia*) urinary infectious bacteria associated with urolithiasis. The fruits were extracted in both water and methanol and the efficacy was tested by the well diffusion method applying 50µL solution/well. The fruit extracts were analysed for the phytochemical screening, qualitative analysis, ICPMS and HPTLC analysis. According to the findings, both extract exhibited an inhibitory activity against the selected gram positive and gram negative bacteria. Both Aqueous and methanol extract has the antibacterial activity with maximum zone of inhibition of 17mm and 20.5mm against *Pseudomonas aeruginosa* respectively. The extracts were reported with phytochemicals like flavanoids, saponins, tannins, carbohydrate, alkaloids, terpenoids, phenol and plobatannin, coumarin and cardiac glycosides and most of them are claimed with antibacterial action. On the basis of the present findings the fruit extracts of *N. cadamba* might be used as a good candidate for preventing the growth of urinary infectious bacteria associated with struvite stones and the antibacterial activity of the fruit extract is attributed by the presence of various phytochemicals.

Keywords: Urolithiasis, antibacterial activity, struvite stones, phytochemical screening, ICPMS, HPTLC

INTRODUCTION

Struvite stones (magnesium ammonium phosphate crystals) are caused by urinary infections with urease producing organisms and contribute 30% of kidney stones. These are fast growing stones remain in the naturally occurring cavities in kidney and form a 'stag horn' appearance. Urinary infections lead to the precipitation of struvite crystals hence the stone are named 'infectious stones'. Presence of renal calculi (stones), indwelling of catheters, immune suppression, renal failure and obstructions results in urinary infection. Although some chronic urinary infections enhance urolithiasis and the presence of stone in the kidney in turn leads to chronic urinary infections (Backman *et al*., 1980). Usually the bacterial infections induce the formation of crystal (Thomson and Stamey; 1973) by increasing the urinary pH and ammonia. In severe conditions, struvite stones cause squamous metaplasia and results in squamous cell carcinoma of renal collecting system. Sometimes the bacteria may reach deep into the stone hence long term antibiotic therapy is necessary for the eradication of microbes. Treatment failure and development of antimicrobial resistance are the serious complications more common associated with urinary infections.

In alkaline state, urine contains precipitated mineral deposits of calcium, magnesium phosphate and calcium carbonate which result in the formation of calcium phosphate and calcium carbonate stones (Chute and Suby; 1943). Phosphate is less soluble at alkaline pH, so phosphate precipitates with ammonium products, yielding magnesium ammonium phosphate, the struvite stones. As the bacteria that produce urease which remain in urine and within stone, they continue to produce urease enzyme, cleave urea and large stones may develop quite rapidly and fill the spaces of the kidney. Most of the bacterial infections are caused by the urea splitting bacteria like *Proteus, Pseudomonas, Klebsiella, Staphylococcus, E. coli* and *Mycoplasma*, which are capable of producing urease enzymes (Griffith *et al*., 1976). *E. coli, Pseudomonas, Streptococcus, Staphylococcus* and *Ureaplasma urealyticum* are reported from the infected stones. Studies revealed that the urea metabolism also damages the glycosaminoglycan layer covering the renal urothelial cells, which provide the biofilm formation and mineral nucleation (Griffith and Osborn; 1987).

Plants are the valuable source of novel antibacterial and renal decrystallizing agents. They are effective in preventing and controlling of nephrolithiasis through different mechanisms. Infections play a major role in nephrolithiasis. So plant extracts with antibacterial activity was reported to have antilithiatic activity. Crystal nucleation, growth, aggregation and crystal retention are the different stages in the formation of renal calculi. Most of the plant based therapies affect any one of the four stages and the antilithogenic property of many plant remedies are attributed to the antimicrobial property (Surendra *et al*., 2011). Antinephrolithiatic action of the fruit extract was already been reported by the same author in earlier studies and the results showed the presence of infectious stones along with calcium oxalate stones from the urine samples of experimental rats (Prathibhakumari and Prasad; 2012). The aim of the present study is to examine the efficacy of aqueous (AFNC) and methanol (MFNC) fruit extracts of *Neolamarckia cadamba* against urinary

infectious bacteria associated with nephrolithiasis.

MATERIALS AND METHODS

Collection and Extraction of plant material:

The fruits of *N. cadamba* were collected from the Botanical Garden, Kerala University Campus, Kariavattom (8^0 37′ 36N, $76^0$50′ 14E), Thiruvananthapuram. The voucher specimen was deposited in the herbarium of Department of Botany, Kariavattom for further reference (Voucher No: KUBH 5811). Dried and powdered plant material was subjected to soxhlet extraction using water and methanol as solvents and concentrated using rotary vacuum evaporator.

Phytochemical screening:

freshly prepared fruit extracts of water, methanol, ethanol, chloroform and petroleum ether of fruits of *N. cadamba* were subjected to preliminary phytochemical investigation for the detection of phytochemicals such as flavanoids, saponins, tannins, carbohydrates, coumarins, quinines, glycosides, alkaloids, steroids, terpenes, cardiac glycosides, oxalate, vitamin C and phenols using standard methods

Quantitative analysis:

Physicochemical characteristics such as Loss on drying, Ash values, total ash value, acid insoluble ash, Extractive values like Water soluble extractive value and Alcohol soluble extractive values were analysed using standard procedures.

ICP-MS analysis:

All the reagents used for the ICP-MS analysis were of suprapur grade (Merck, USA) and high purity water from Milli-Q water purification system (Thermo Scientific, Barnstead, Smart 2 pure) was used for dilution and preparation of sample.

HPTLC analysis:

HPTLC finger print analysis was carried out using the methodology of Wagner *et al*. (1996) and Harborne (1998). The fruit extract was dissolved in HPLC grade methanol and spotted the sample. The selected solvent system for the fruit extract was toluene: chloroform: ethanol in the ratio of 4:4:2. 5µl and 10 µl, 15µl and 20µl of the extract were separately spotted on silica gel 60 F254 with precoated aluminium TLC plate support (20cmx10cm). CAMAG Linomat 5 automatic sample spotter was used as the spotting device with inert gas providing delivery speed of 150nl/s from the syringe. The samples were spotted in the form of bands with bandwidth of 8mm using Hamilton syringe with 100µl sample in the precoated TLC sheet with the

help of Linomat 5 applicator attached to CAMAG HPTLC system. After the application of sample, the plates were kept in CAMAG twin glass tank saturated with the mobile phase for 15 minutes. Air dried plates were sprayed with Anisaldehyde sulphuric acid reagent and the photo documentation of the extract was observed under ultra violet (UV) and visible light at 200nm, 254nm, 366nm and 560nm.

Test organisms:

Escherichia coli (MTCC 2968), *Pseudomonas aeruginosa* (MTCC 3542), *Staphylococcus aureus* (MTCC 3160), *Proteus mirabilis* (MTCC 3310) and *Klebsiella pneumonia* (MTCC 3040) were the clinical strains obtained from MTCC microbial culture collection, Chandigarh were used for the antibacterial assay.

Antimicrobial assay:

The antibacterial activity of both aqueous and methanol fruit extracts of *N. cadamba* was tested against both gram positive and gram negative bacteria. The inoculums of microorganism were prepared from bacterial culture. Detection of antibacterial activity was done by well diffusion method (Kirby *et al*., 1966). Antibacterial activity was evaluated by measuring the diameters of zone of inhibition (ZOI) around each well. For each organism, triplicates were carried out and the average inhibition zone diameter was determined.

RESULTS

The phytochemical screening results revealed that aqueous extract was found to contain the phytochemicals like flavanoids, saponins, tannins, carbohydrate, alkaloids, terpenoids, phenol and plobatannin whereas in methanol extract the reported phytoconstituents are flavanoid, tannins, carbohydrate, coumarin, alkaloids, terpenoids, cardiac glycosides and phenols (Table 1). However, coumarin, alkaloids, steroids, terpenoids and cardiac glycosides are present in the ethanol fruit extract. But tannin, carbohydrate, alkaloids and terpenoids showed positive test results for chloroform extract whereas the petroleum ether extract of fruit revealed the presence of alkaloid, terpenoids and vitamin C. The reported total ash value and acid insoluble ash value are 3.208% and 0.939%. The loss on drying at 105˚C was found to be 19.15%. The extractive values such as water soluble extractive value and alcohol soluble extractive value was also determined (Table 2). The water soluble extractive values obtained from fruit of *N. cadamba* is 16.06% and the observed alcohol soluble ash value is 9.21%. The yield of the aqueous fruit extract was found to be 36.25%.

The elemental analysis of fruits of *N. cadamba* revealed the presence of various elements such as Mg, Al, Cr, Mn, Fe, Co, Cu, Zn, Cd, Pb and Se. The concentration of magnesium (Mg) in the fruits of *N. cadamba* is 1356.24ppm. Our result indicates that the Mg, Aluminium (Al), Manganese (Mn) concentration in the plant material is within the permissible limit set by WHO. The amount of Iron (Fe) content in the fruit is

344.88ppm. Maximum limit recommended by WHO for Fe is 200ppm hence, in the present study the plant material showed more Fe concentration. Selenium (Se) concentration has not detected by the ICP-MS analysis (Table 3).

Sl.no	Phyto Chemicals	Screening Tests	Solvent systems				
			AE	ME	EE	CE	PEE
1	Flavanoids	Ferric chloride test	-	+	-	-	-
		Alkaline reagent test	+	+	-	-	-
		Lead acetate test	-	+	+	-	-
2	Saponins	Foam test	+	-	-	-	-
3	Tannins	Gelatin test	+	+	-	-	-
		Ferric chloride test	-	+	-	-	-
		Braymer's test	+	+	-	+	-
4	Carbohydrate	Benedict's test	+	+	-	+	-
		Iodine test	+	+	-	-	-
5	Coumarins		-	+	+	-	-
6	Quinines		-	-	-	-	-
7	Glycosides	Fehlings test	-	-	-	-	-
8	Alkaloids	Dragendorff's reagent test	+	+	-	-	-
		Wagners test	+	+	+	+	+
		Hagers test	+	+	-	-	-
9	Steroids		-	-	+	-	-
10	Terpenoids	Salkowki's test	+	+	+	+	+
11	Cardiac glycosides	Keller killiani's test	+	+	+	-	-
		Bromine water test					
12	Phenol	Ferric chloride test	+	+	-	-	-
13	Phlobatannins	Precipitation test	+	-	-	-	-
14	Anthraquinones		-	-	-	-	-
15	Oxalate		-	-	-	-	-
16	Protein	Biuret test	-	-	-	-	-
17	Aminoacids	Ninhydrin test	-	-	-	-	-
18	Vitamin C	DNPH test	-	-	-	-	+

Table 1: Phytochemical screening of fruits of *N. cadamba*

+ = Presence, - = Absence, AE = Aqueous extract, ME = Methanol extract, EE = Ethanol extract, CE = Chloroform extract, PEE = Petroleum ether extract.

Sl. no	Constituents	% value
1	Total ash	3.208%
2	Acid insoluble ash	0.93%
3	Alcohol soluble extractive value	9.21%
4	Water soluble extractive value	16.06%
5	Loss on drying at 105˚C	19.15%
6	Yield (aqueous extract)	36.25%
7	Yield (methanol extract)	20.26%
8	Volatile oil	Nil

Table 2: Physicochemical characteristics of fruits of *N. cadamba*

Elements	Concentration (ppm)
Magnesium (Mg)	1356.24
Aluminium (Al)	540.5
Chromium (Cr)	9.52
Manganese (Mn)	73.09
Iron (Fe)	344.88
Cobalt (Co)	0.63
Copper (Cu)	26.19
Zinc (Zn)	25.29
Cadmium (Cd)	0.45
Lead (Pb)	5.2
Selenium (Se)	Not detected

Table 3: Elemental concentration in fruits of *N.cadamba*

The HPTLC finger print profiling of *N. cadamba* scanned at 254nm showed 8 peaks at 10µl concentrations (Table 4). The Rf value of 0.14 showed maximum concentration of phytocomponent of 33.62% and remaining components were found very less in quantity as the percentage area is decreased. The chromatogram, peaks and photo documentation at 254nm are shown in Figs. 1, 2 and 3. The result from the finger print profile of the fruit extract scanned at 540nm was observed with 15 peaks at 10µl concentration (Fig. 4 and 5). The Rf value of the extract ranged from 0.04 to 0.92 (Table 5). The maximum concentration of phytoconstituents (14.50%) was recorded at the Rf value of 0.40. The photo documentation of the extract at 540 was shown in Fig. 6.

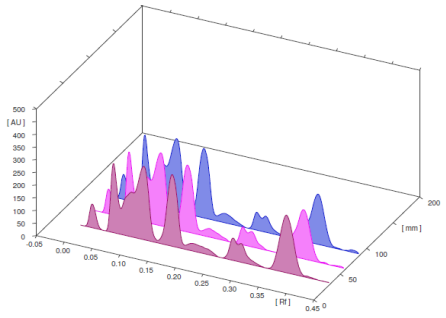

Figure 1: Chromatogram of *N. cadamba* fruit extract at 254nm

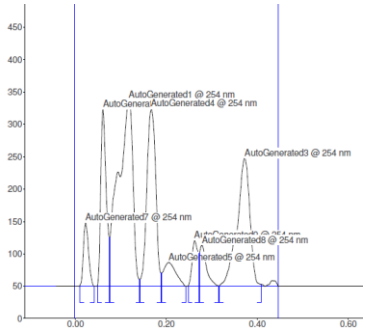

Figure 2: Chromatogram of the fruit extract of *N. cadamba*at254nm (10µl)

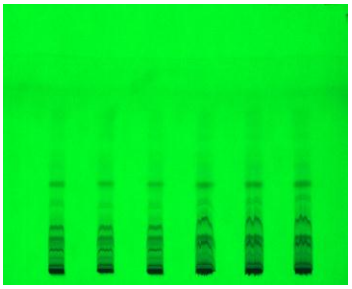

Figure 3: HPTLC photo documentation at 254nm

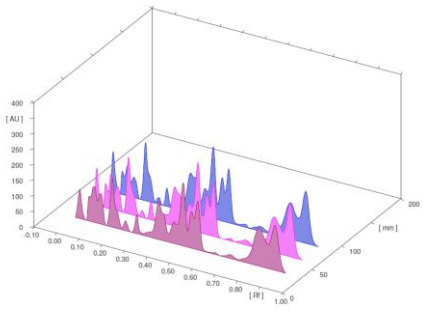

Figure 4: Chromatogram of *N. cadamba* fruit extract at 540nm

Peak	Start Rf	Max Rf	End Rf	Area (%)
1	0.01	0.02	0.04	4.03
2	0.05	0.06	0.08	12.10
3	0.08	0.12	0.14	33.62
4	0.14	0.17	0.19	19.58
5	0.19	0.21	0.24	3.65
6	0.25	0.26	0.27	3.34
7	0.27	0.28	0.32	3.42
8	0.32	0.37	0.41	20.26

Table 4: Peak list and Rf values of chromatogram at 254nm (10µl)

Peak	Start Rf	Max Rf	End Rf	Area (%)
1	0.00	0.02	0.04	4.09
2	0.05	0.06	0.07	1.78
3	0.07	0.08	0.10	4.61
4	0.10	0.11	0.13	3.06
5	0.14	0.16	0.21	9.74
6	0.21	0.22	0.24	0.75
7	0.24	0.26	0.28	1.91
8	0.29	0.36	0.40	14.50
9	0.41	0.43	0.44	4.52
10	0.44	0.46	0.48	12.15
11	0.48	0.50	0.51	6.38
12	0.52	0.53	0.57	7.90
13	0.62	0.67	0.70	1.47
14	0.70	0.80	0.84	14.36
15	0.84	0.87	0.92	12.78

Table 5: Peak list and Rf values of chromatogram at 540nm (10µl)

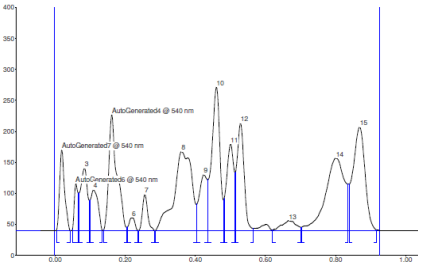

Figure 5: Chromatogram of the fruit extract of *N. Cadamba* at 540nm (10µl)

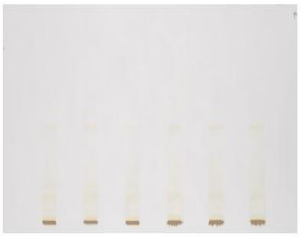

Figure 6: HPTLC photo documentation at 540nm

Bacteria selected	*N. cadamba* fruit extracts			
	Aqueous		Methanol	
Escherichia coli	+	++	+	+++
Pseudomonas aeruginosa	+	++	+	+++
Staphylococcus aureus	+	++	+	++
Proteus mirabilis	-	-	-	
Klebsiella pneumoniae		+	+	++

Table 6: Antibacterial activity of fruit extract of *N. cadamba* on selected gram positive and gram negative bacteria

(-), no zone of inhibition (ZOI). (+), 6-10mm in diameter of ZOI. (++), 11-15mm in diameter of ZOI. (+++), ≥16mm in diameter of ZOI

The present study is focused on the antibacterial activity of both aqueous and methanol fruit extract of *N. cadamba* against various pathogenic microorganisms. *E. coli, P. aeruginosa, S. aureus, P. mirabilis* and *K. pneumoniae* are the bacterial strains tested for evaluating the antibacterial activity. The zone of inhibition (ZOI) indicates the effectiveness of the plant extracts in inhibiting the growth of bacteria (Table. 6). Generally, larger the ZOI, the more sensitive the bacterium to the plant extracts.

The aqueous fruit extract in higher concentration (400mg/kg) was found to have maximum zone of

inhibition against *E. coli* than the lower concentration. The results revealed that high concentration of aqueous extract have maximum antibacterial activity against *E. coli* than low concentration of the extract (Fig. 7). The reported zone of inhibition in methanol fruit extract at 400mg/kg was 19.5±2.5mm in diameter, which was the maximum zone of inhibition and most dominant antibacterial activity. All doses of both plant extracts had antibacterial activity against *E. coli*. The aqueous fruit extract of *N. cadamba* was found to be more resistant to the bacterium, *Staphylococcus aureus*. Among both concentrations of aqueous extracts, highest inhibitory activity against *S. aureus* was observed in high concentration and the observed maximum zone of inhibition in 400mg/kg was 13±1mm. Both types of fruit extracts manifested the antimicrobial activity against gram positive bacteria, *S. aureus.* Dose dependent increase in zone of inhibition was also observed in both aqueous and methanol fruit extracts against *P. aeruginosa.* The observed 10±2mm diameter of ZOI in low dose of aqueous extract was found to be increased (17±1mm) at a dose of 400mg/kg.

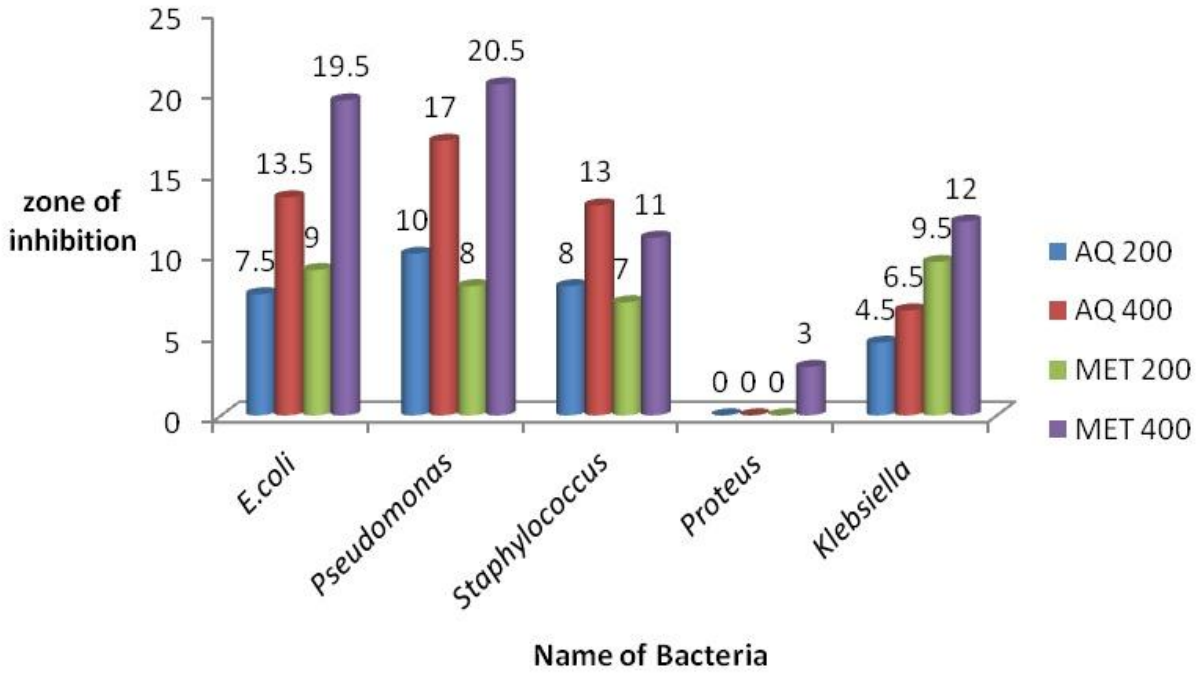

Figure 7: Effect of fruit extract of *N. cadamba* on selected pathogenic bacteria

Proteus mirabilis was resistant to both extract except high concentration (400mg/kg) of methanol fruit extract of *N. cadamba*. Different concentration of aqueous and alcohol fruit extract of *N. cadamba* was found to be resistant against *Klebsiella* species. Aqueous fruit extract exhibited an inhibition zone of 4.5±0.5 and 6.5±0.5mm in the low concentration and high concentration respectively. The results showed that the antibacterial activity of the fruit extract was increased with increasing concentration of the extract. The extract showed prominent antibacterial activity against gram negative bacteria, *K. pneumoniae* and for

Proteus species, the ZOI was found to be 3±0.01mm even in the high dose of the methanolic fruit extract.

If the ZOI exceeds 15mm in diameter, the antimicrobial activity is considered as very good and the diameter is between 15mm and 8mm, the activity of the extract is considered average. The antibacterial activity is weak when the diameter of zone of inhibition is below 8±0.01mm. Very good (+++) antibacterial activity was noticed against *E. coli* at a concentration of 400mg/kg in the methanol extract of *N. cadamba* fruit. But *P. aeruginosa* exhibited a very good (+++) antibacterial activity in the high concentration of both aqueous and methanol fruit extracts. The average (++) antimicrobial activity was observed with high concentration of aqueous and low concentration of methanol extracts against *E. coli*. Low concentrations of both aqueous and methanol extracts have average antibacterial potential against *P. aeruginosa*. Maximum antibacterial activity against *E. coli*, *P. aeruginosa* and *K. pneumonia* was observed in high concentration of methanol extract but highest antibacterial activity against *S. aureus* was noticed against high concentration of the aqueous fruit extract. From the above mentioned results, it is clear that the fruit extract of *N. cadamba* had antibacterial action against both gram positive and gram negative bacteria.

DISCUSSION

The successful screening of the phytochemicals depends on the type and number of solvents used for extraction process. Of the eighteen phytochemicals screened, twelve are present in various solvents. Most of these secondary metabolites have curative activity against clinical problems in humans like diuretics, spasmodic, diarrhoea, dysentery and menstrual disorders (Nisar *et al*., 2011). The results indicated that fruits of *N. cadamba* possess diverse phytochemicals and the presence of these phytochemicals could be responsible for the therapeutic property of fruits of *N. cadamba.* The acid insoluble ash value (0.93%) of fruit reported very little amount of inorganic compound. Loss on drying at 105˚C is the measure of moisture content of the plant material. The high moisture content of the plant material enhances the growth of bacteria, fungi and yeast colonies and less amount of moisture content prevent the growth of these colonies (Mulla and Swamy; 2010). The percentage yield was found to be high (36.25%) in aqueous fruit extract when compared with methanol extract and the yield of the plant material varied according to the solvent of extraction (Tarachand *et al*., 2012).

The data revealed that most of the phytoconstituents were readily dissolved and extracted in aqueous extract than methanol extract. The highest water soluble extractive value (16.06%) of fruit could be due to the presence of sugars, acids and inorganic compounds when compared with alcohol extractive values. The alcohol soluble extractive values indicated the presence of phenols, steroids, glycosides, flavanoids and alkaloids (Meena *et al*., 2010). The presence of these secondary metabolites is the reason of reported 9.21% of alcohol soluble extractive value of plant material. The results of extractive values indicated that most of the constituents are soluble in water than alcohol. The physicochemical and phytochemical screenings of plant

material are used as diagnostic tools for the standardization and evaluation of plant material. In the present study, large arrays of bioactive secondary metabolites and diverse elements are reported from the fruit extracts of *N. cadamba* in various solvents lighten the therapeutic property of the plant.

The HPTLC analysis of the fruit extract of *N. cadamba* reported the presence of different bioactive compounds as peaks in the densitogram of HPTLC and are represented by corresponding retention factor (Rf) value. Hence, the isolation, identification and purification of these phytocompounds in accordance with the Rf value can be useful for the formulation of new drugs (Sunil *et al*., 2013). In the recent times plants are being classified based on the anatomical, biochemical and molecular markers. Here, HPTLC finger printing profile is used as a phytochemical marker and to estimate the genetic variability in the plant population. The HPTLC fluorescence image coupled with the scanning profile provide adequate information for the identification, assessment and comparison of major active phytocompounds in the plant sample for their use in medicinal preparations (Kamboj and Saluja; 2011). The results showed that alkaloids found its maximum percentage area in HPTLC chromatogram than other phytochemicals. The present HPTLC study provides a basic information tool useful for the isolation, purification and identification of marker compounds of fruits of *N. cadamba* and served as an effective raw material for drug formulation.

The screening of the fruit extract of *N. cadamba* against pathogenic micro organisms were carried out in two different concentrations using water and methanol as solvents. Among two different extracts used, the methanol extract of fruits of *N. cadamba* reported the maximum zone of inhibition of all gram negative bacteria such as *E. coli* (19.5±2.5mm), *P. aeruginosa* (20.5±0.5mm), *P. mirabilis* (3±0.01mm) and *K. pneumoniae* (12±0.01mm). The maximum antibacterial activity against tested organisms was reported by high concentration of fruit extract (400mg/kg). The results clearly revealed that the aqueous fruit extract also possesses antibacterial activity but the diameter of ZOI was found to be low when compared to the methanol extract and the results are in agreement with the previous works, which showed that most of the compounds in plants with antibacterial potential are reported in methanol (Chandrasekaran and Venkatesalu; 2004) and water extract had low antibacterial activity (Ashafa *et al*., 2008).

Studies reported that alcohol is the solvent for complete extraction of compounds with different polarities for plant extraction process (Evan, 1996; Eloff, 1998) whereas the aqueous extract contains only some of the less polar compounds. The solubility of the phytoconstituents varied depending on the type and polarity of the solvent used (Marjorie, 1999). This may be the reason of higher antibacterial activity against pathogenic micro organisms in methanol extract of fruits of *N. cadamba* than the aqueous extract. The results of the antibacterial activity by aqueous extract provides the scientific basis for the use of the plant in most of the traditional treatments against various diseases in which traditional medical system uses water as a solvent for preparing the decoctions.

The low antibacterial activity of the extracts against *P. mirabilis* bacteria might be due to the

resistance of the tested pathogenic bacterial strains over the fruit extract. Only the high concentration of the methanol extract produced the zone of inhibition of 3±0.01mm. It revealed that *P. mirabilis* is more resistant to both concentration of aqueous fruit extract and low concentration of the methanol extract. The difference in antibacterial activity of both aqueous and methanol extracts may be attributed to the difference in the peptidoglycan layer of bacterial cell wall. An essential function of the peptidoglycan layer is to protect against antibacterial agents like antibiotics, chemicals and degrading enzymes (Thomas *et al*., 2010).

Generally, gram negative bacteria are multi-resistant against antibiotics. So the antibacterial activity in methanol extract of *N. cadamba* fruit against these bacteria is promising for developing antibiotics. However, the aqueous extract showed the highest inhibitory activity (13±1mm) over the growth of gram positive bacteria, *S. aureus*. Gram positive bacteria have peptidoglycan layer and lack an outer lipid membrane (Leach, 1986). This helps the extract to penetrate and inhibit the growth of gram positive bacteria. The efficacy of the fruit extract against both gram positive and gram negative bacteria may be the indicative of the presence of broad spectrum of antibiotic compounds in the plants as reported by Siddhuraju and Becker (2003).

Several studies revealed that the presence of bioactive compounds in plants inhibit the growth of strains of pathogens (Meng *et al*., 2000). The phytochemicals in the fruit extract might be the reasons of antibacterial activity. These components may act individually or synergistically for the reported antibacterial activity. Studies suggest that sterols, alkaloids and tannins have been reported with antibacterial activity (Leven *et al*., 1979). The demonstrated antibacterial activity of the *N. cadamba* fruit extract in the present study could be due to the presence of broad spectrum antibiotics or several metabolic toxins as observed by Sharma *et al*. (2012). The present study revealed that both aqueous and methanol fruit extracts have strong antibacterial activity against the urea splitting infectious bacteria which cause aggregation and growth of crystals in kidneys such as *E. coli, Pseudomonas, Klebsiella* and *Staphylococcus*.

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