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ISSN: 0975-7538
Research Article

A quantitative assessment of bioactive phytochemicals of *Nerium indicum*: An ethnopharmacological herb

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ABSTRACT

Phytochemicals fall under various classes of chemicals which are solely derived from plants. Some of these plants possess immense medicinal and therapeutic value for which the modern pharmaceutical, cosmetic and food industry aims towards utilizing the natural plant based resources. These phytochemicals have lesser side effects as believed than the conventional synthetic chemicals and also are more economic. From ages, extracts from various plants are used as traditional medicines all over the world. These extracts, rich in phytochemicals, may provide protection from various diseases and also may develop the resistance against some diseases. *Nerium indicum* is one of the traditional medicinal plants used all over the world and especially in Indian subcontinent and China. In the present study we have documented the presence or absence of different phytochemicals like tannin, phlobatannin, cholesterol, terpinoid, glycoside, phenolics, flavonoid, steroid, anthraquinone, saponin, carbohydrate, alkaloid and protein in leaf, stem separately and root apart from the whole plant part of *N.indicum*. We also quantified the amount of saponin, alkaloid, flavonoid, tannin, riboflavin, ascorbic acid, thiamine, soluble sugar, protein, lipid, total moisture and ash content in different parts of the plant. All the methods followed are standard biochemical and spectrophotometric procedures for detection and quantification of phytochemicals. This may lead to open a new vista regarding the medicinal value of *N. indicum.*

Keywords: Nerium; phytochemical; flavonoid; phenol; pharmaceutical; herbal medicine; vitamin

INTRODUCTION

Plant derived chemical substances have some beneficial effect on the body (Akinmoladun AC et al. 2007). Certain beneficial phytochemicals scavenge harmful free radicals, work as immunomodulators and may have anti-inflammatory, anti-cancer and antimicrobial activities and repair cellular damage (Sun J et al. 2002; Chakraborty S et al. 2004). Though ethnopharmacology has recently came to the lime light, but for time immemorial, people have cured themselves by using local plants. The World Health Organization (WHO) has estimated that more than 80% population of the world relies primarily on plant based traditional remedies (Akerlee O, 1996). In recent times, ethnomedicines have become the focus of pharmaceutical research. The synthetic drugs and antibiotics are sometimes associated with adverse side effects which include hypersensitivity, immunosuppression and allergic reactions. Shift of faith from the modern medicine to alternative and complementary medicines (CAM) is seen among huge population worldwide. With the emerging cases of antibiotic resistance in bacteria, there is a constant need for new and effective therapeutic agents from plants. Thus, the plant derived bioactive phytochemicals have drawn the main attention as a source of CAM.

Nerium indicum Mill locally known as Sheth Karabi (Bengali) and Kaner (Hindi) is an erect, smooth, evergreen shrub belonging to the Apocynaceae family. Different parts of *N. indicum* (white flower type) are being used as traditional medicine in different parts of the world especially in India (Dasur JF. 1985) and China (Ding K et al. 2003). Polysaccharides of *N. indicum* have shown anti-tumor (Lin ZB and Zhang HN, 2004), immune-stimulating (Leung HY. et al 2006) and neuroprotective effects (Yu MS and Lai SW, 2004). *N. indicum* have proved itself as a potent antimicrobial agent (Hussain MA and Goris MS, 2004). All the parts of the plant is said to possess anticancer properties (Abe F and Yamauchi K, 1992). But, studies indicating a clear phytochemical profile of this plant have not been done yet. Therefore, the aim of the present study was to investigate the presence of different types of phytochemicals both qualitatively and quantitatively that will lead us to a clear phytochemical status of *N. indicum*. Three major parts of the plant like leaf, stem and root were examined for the presence of different phytochemicals such as tannin, phlobatannin, cholesterol,

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Received on: 12-07-2012
Revised on: 18-09-2012
Accepted on: 20-09-2012

terpinoid, glycoside, phenolics, flavonoid, steroid, anthraquinone, saponin, carbohydrate, alkaloid and protein. The quantitative estimation of saponin, alkaloid, flavonoid, tannin, riboflavin, ascorbic acid, thiamine, soluble sugar, protein, lipid, total moisture and ash content in all three parts of the plant have also been done.

MATERIAL AND METHODS

Chemicals and reagents: All solvents and materials were obtained from Sisco Research Laboratories Pvt. Ltd. (Mumbai. India), unless otherwise indicated. Analytical grade H_2SO_4, chloroform, acetic acid, ethyl acetate, trichloroacetic acid (TCA), diethyl ether and isoamyl alcohol were purchased from Merck Specialties Pvt. Ltd. (Mumbai, India). Ferric chloride, α-napthol, sodium sulphate, bovine serum albumin (BSA) tannic acid, gallic acid, thiamine, riboflavin and n-hexane were obtained from HiMedia Laboratories Pvt. Ltd. (Mumbai, India). HCl was supplied by Thomas Baker (Mumbai, India).

Sample collection: White flowered variety of *N. indicum* was collected from the campus area of the University of North Bengal, India. The plant was identified by Taxonomist Prof. A. P. Das of Department of Botany, University of North Bengal and the voucher specimen was stored at the Botany Department Herbarium, University of North Bengal with an accession number of 9618.

Sample preparation: The whole plant was separated into three major parts: leaf, stem and root. The parts were washed properly first with tap water and then with double distilled water to remove dirts. The parts were then shade dried at room temperature for two weeks and grinded to powder. The powder was passed through a 0.5 mm metallic mesh. The resultant crude fine plant powder was used for the phytochemical investigations according to the standard chemical tests (Brain KR and Turne TD, 1975; The Indian pharmacopoeia. 1996; Khandelwal KR. 2008; Gokhale SB and Kokate CK, 2008).

Qualitative tests

10 g of crude dried plant was taken in a 250 ml conical flask and 100 ml of double distilled water was added to it. The solution was mixed on a magnetic stirrer for 10 hr. The mixture was filtered through Whatman filter paper number 1 (150 mm) and the filtrate was used for the following phytochemical tests.

Tannin: 10 ml of aqueous extract was mixed with few drops of 0.1% $FeCl_3$ solution. Blue-black precipitate formation upon addition of ferric chloride solution indicates the presence of tannin.

Phlobatannin: 10 ml of aqueous extract was taken in a boiling tube and 2 ml of concentrated HCl was added to it. The mixture was boiled for 1 minute. Caution was taken so that the hot solution does not bump out of the boiling tube. Deposition of red precipitate is the indication of presence of phlobatannins.

Carbohydrate: 2 ml of aqueous extract was mixed with 2 ml of Molish's reagent (5% α-napthol in absolute ethanol) and shaken vigorously to mix properly. 2 ml of concentrated H_2SO_4 was added carefully by means of a pipette along the wall of the test tube. Formation of reddish-violet ring at the junction of two liquids indicates the presence of carbohydrates.

Proteins: 2 ml of aqueous solution was taken in a test tube and 1 ml of 40% NaOH solution was added to it. The solution was mixed properly and 1-2 drops of $CuSO_4$ solution was added to it. Change of coloration of the solution to violet indicates the presence of peptide linkage in a solution which in turn is an indication of the presence of proteins.

10 g of crude dried plant powder was taken with 100 ml of 70% methanol in a 250 ml conical flask. The mixture was mixed in a magnetic stirrer for 10 hr in room temperature and filtered through Whatman filter paper number 1. The filtrate was used for the following phytochemical tests.

Terpinoid: 5 ml filtrate was mixed with 2 ml of chloroform. 3 ml of concentrated H_2SO_4 was added to the solution slowly along the wall of the test tube. Care was taken not to stir the solution in the test tube. Reddish-brown coloration formed at the junction of two liquid phases indicates the presence of terpinoids.

Glycoside: 5 ml of the methanol extract was taken in a separate test tube and 2 ml of Glacial acetic acid containing 2% $FeCl_3$ solution was added to it. 1 ml of concentrated H_2SO_4 was added slowly along the walls of the test tube. Formation of a brown ring at the interphase of two liquid notifies the presence of glycoside.

Steroid: 5 ml of methanol extract was treated with 0.5 ml of anhydrous CH_3COOH and was cooled on an ice bath for 15 min. 0.5 ml of chloroform was added to the solution. 1 ml of concentrated H_2SO_4 was poured along the walls of test tube carefully by means of a pipette. At the separation level of two liquids, a reddish-brown ring was formed, as an indication of the presence of steroids.

Cholesterol: 2 ml of ethanolic extract was separately mixed with 2 ml of chloroform. 10-12 drops of acetic acid anhydride was added to the tube and shaken. 2 drops of concentrated $H_2SO_4$was added to it. Change of reddish-brown coloration to blue-green on addition of H_2SO_4 indicates the presence of cholesterol.

Alkaloid: 2 ml of filtrate was taken in a test tube and 2 ml of 2N HCl was added to it. The solution was shaken vigorously to mix and kept aside for five minutes. Aqueous phase was separated from the two liquid phases and few drops of Mayer's reagent ($HgCl_2$ + KI in water) was added to it and shaken to observe the formation of creamy colored precipitate.

Phenol: 10 ml of ethanol extract was treated with 4-5 drops of 2% $FeCl_3$ solution. Change of coloration of the solution indicates presence of phenolics.

Flavonoid: 2 g of crude powdered plant sample was heated with 10 ml of ethyl acetate over a water steam bath for a period of 5 minutes. The solution was filtered through Watman filter paper number 1. 4 ml of the filtrate was mixed with dilute ammonia solution (10%) and shaken vigorously. Yellow coloration of the solution indicates the presence of flavonoids.

Anthraquinone: 0.5 g of crude plant powder was taken in a 100 ml conical flask and 20 ml of benzene was added to it. The mixture was stirred in a magnetic stirrer for 4 hr and filtered. 10 ml of filtrate was mixed with 0.5 ml ammonia solution and mixed properly. Presence of violet color at the layer phase indicates presence of anthraquinones.

Saponin: 0.5 g of powdered plant material was boiled with 15 ml of double distilled water in a boiling water bath. Formation of intensive froth is the indication of presence of saponin.

Quantitative tests

The quantitative estimation of different phytochemicals are performed according to various standard methods.

Alkaloid determination: The assay was performed according to a standard method (Obadoni BO and Ochuko PO, 2001; Harborne JB. 1983). 5 g of sample powder was taken into a 250 ml beaker and 250 ml of 20% CH_3COOH in ethanol was added to it. The mixture was shaken on a magnetic stirrer for 10 hs at room temperature. The solution was filtered through Whatman filter paper and the resultant was placed on a hot water bath (60^o C) until the extract volume turns ¼ th of its initial volume. Concentrated NH_4OH was added to it drop wise which forms thick precipitate. NH_4OH was added until the precipitate formation was complete. The whole solution was allowed to settle down. The precipitate was collected by filtration, dried in an oven and weighed.

Flavonoid determination: A standard method (Boham AB and Kocipai DC, 1994) was followed with slight modifications to quantify the total Flavonoid content. 10 gm of plant powder was taken in a flask and 100 ml of 70% methanol was added to it. The mixture was stirred using a magnetic stirrer for 3 hours and filtered through Whatman filter paper Number 1. The remaining powdered material was re-extracted once again with 70% methanol and filtered in similar way. Both the filtrates were mixed and transferred into a crucible and evaporated to dryness over a water bath of 60^0 C and weighed.

Estimation of saponin: Saponin estimation was done according to a slightly modified standard method (Nahapetian A and Bassiri A, 1974). 10 g of sample powder was taken in a flask and 100 ml of 20% ethanol was added to it. The mixture was heater over a hot water bath of 55^0 C for a period of 5 hr with continuous stirring. The mixture was filtered and the supernatant liquid was separated. The solid residue was mixed with another 100 ml 20% ethanol and as previous 5 hr. The solution was filtered and the extract was mixed with the previously stored extract. The combined extract was placed on a hot water bath of 90^o C and heated till the volume of the extract was reduced to 20% of its initial volume. The concentrate was transferred into a 250 ml separating funnel and 10 ml of diethyl ether was added to it and shaken vigorously. After the solution settles down the aqueous layer was separated carefully into another flask and the ether layer was discarded. The purification process was repeated again. 60 ml of n-butanol extracts were washed twice with 10 ml of 5% aqueous NaCl solution. The remaining solution was heated in a water bath at 50^o C until the solvent evaporates and the solution turns to semi dried form. The sample was then dried in an oven into a constant weight. The saponin content was calculated by the following equation:

$$\text{Percentage of saponin} = (W_{EP} / W_S) * 100$$

Where, W_{EP} = Weight of oven dried end product; W_S = Weight of powdered sample taken for test.

Tannin determination: The assay was performed according to a previously described standard method (Van-Burden TP et al. 1981). 1 g of crude powder sample was taken in a 100 ml flask and 50 ml of double distilled water was added to it and shaken on a magnetic stirrer for 10 hours at room temperature. It was filtered into a 50 ml volumetric flask and made up to the mark using distilled water. 5 ml of solution was pipetted out in a test tube and 0.008 M $K_4[Fe(CN)_6]$ and 0.1 M $FeCl_3$ in 0.1 N HCl was added to it. The absorbance was measured in sperctophotometer at 120 nm wavelength within 10 minutes. A blank was prepared and read at the same wavelength. A standard was prepared using tannic acid to get 100 ppm and measured.

Determination of riboflavin: The test was performed according to a standard method (Abe F and Yamauchi K, 1992) with slight modifications. 10 g of crude powder was taken in a 250 ml conical flask and 100 ml 50% ethanol was added to it. The mixture was stirred for 10 hr on a magnetic stirrer at room temperature. The solution was filtered and 25 ml of 5% $KMnO_4$ solution was added to it. The mixture was stirred using a glass rod continuously while 25 ml of 30% H_2O_2 was added to it. This was placed on a 80^0 C water bath for 30 min. 5 ml of 40% Na_2SO_4 was added to it and the absorbance was measured at 510 nm using a spectrophotometer. A blank was prepared with all the chemicals except the plant material. The riboflavin content was calculated from a riboflavin standard curve.

Determination of thiamine: A previously standard method was followed with slight modifications

(Poornima GN and Rai VR, 2009) to quantify the thiamine content. 50 g of plant sample was dispersed in 50 ml ethanolic NaOH (20%) and stirred over a magnetic stirrer for 3 hours at room temperature. The resultant was filtered into a 100 ml conical flask. 10 ml of the filtrate was separated out and 10 ml of 2% potassium dichromate solution as added to it. As a result color will develop which was read 360 nm against a suitable blank. The blank contained all except the plant material. The thiamine content was calculated from a thiamine standard curve.

Determination of ascorbic acid: Slightly modified method of Barakat MZ et al. (1993) was followed to estimate the quantity of ascorbic acid. 5 g of powder sample was mixed with 100 ml extraction mixture (TCA : EDTA at 2:1) and stirred on a magnetic stirrer for 3 hr at room temperature. This was transferred to a centrifuge tube and centrifuged at 2000 rpm for 30 min. After centrifugation the supernatant liquid was filtered. 2-3 drops of 1% starch indicator was added to the filtrate and it was titrated against 20% $CuSO_4$ solution until a dark end point is reached.

Determination of total phenols: To estimate total phenol (Obadoni BO and Ochuko PO, 2001) content the test sample needed to be fat free. To prepare fat free sample, 5 g of crude plant powder was mixed with 100 ml n-hexane and defatted using a soxlet apparatus for 2 hr. The resultant was used for determination of total phenols.

For the extraction of the total phenolic content, the fat free sample was boiled with a 50 ml of ether for 15 min. The resultant was filtered and 5 ml of the filtrate was pipetted into a 50 ml flask. 10 ml of double distilled water was added to it. 2 ml of NH_4OH solution and 5 ml of concentrated amyl alcohol was added to the solution with constant stirring. The flask was incubated at room temperature for 30 min for color development. The absorbance of the solution was read using spectrophotometer at 550 nm against a suitable blank. The phenolic content was evaluated from a gallic acid standard curve.

Estimation of total protein: Total protein was estimated according to the method proposed by Lowry OH et al*.* (1951) with slight modifications. Known concentrations of bovine serum albumin was taken as standard and the OD was read at 750 nm using a suitable blank.

Estimation of total lipid: The assay was performed according to a standard method (Jayaraman J. 2011) with slight modifications. 1 g of dried sample was macerated with 10 ml distilled water. To this, 30 ml of chloroform-methanol (2:1 v/v) was mixed thoroughly and the mixture was left overnight at room temperature. 20 ml of chloroform and equal volume of distilled water was added and centrifuged at 1000 rpm for 10 min. After centrifugation three layers were formed, out of which the lower layer was collected which contained chloroform containing lipid. The mixture was kept in an oven for one hour at 50^0 C which resulted in the evaporation of chloroform. Weight of the remaining was taken.

Total sugar estimation: The total sugar content was determined according to Dubois MK et al. (1951) with slight modifications. 50 g of the powdered test sample macerated in a pestle and mortar with 20 ml of ethanol and kept for incubation at 30^0 C for 10 hr. The mixture was centrifuged at 1500 rpm for 20 min and the supernatant was collected separately. To 1 ml of alcoholic extract, 1 ml of 5% phenol solution was added and mixed. 5 ml of concentrated H_2SO_4 was added rapidly with constant stirring. This was allowed to stand for 30 min at room temperature. The solution color changed in to yellow orange and the OD was measured at 490 nm against a blank. The blank standard curve was prepared using known concentrations of glucose. The quantity of the sugar was expressed as mg/g fresh weight of the sample.

Moisture and ash content determination: Moisture and ash content of different parts of the plant was estimated by subjecting specific amount of sample to 90^o C for 12 hr in an oven and at 400^o - 450^o C in a furnace for 5 min respectively. The resultant weight was calculated for moisture and ash content estimation respectively.

Statistical analysis: All the experiments were performed three times and the data were reported as the mean ± SD of three measurements. Statistical analysis was performed using KyPlot version 2.0 beta 15 (32 bit).

RESULTS AND DISCUSSION

N. indicum is being used as traditional medicine for ages and the result of its phytomolecular screening directly correlates with the facts. We detected the presence of steroids, phenolics, flavonoids, saponins, tannins, glycosides, terpinoids and other phytochemicals in the different parts of the plant which are essential constituents of herbal medicines and also estimated the quantity of some of the phytochemicals and phytonutrients.

Different parts of *N. indicum* showed either presence or absence of different phytochemicals. All the results are listed below in the Table 1 and Table 2.

Quite a high percentage of alkaloid has been detected in all the parts of the plant among which the root (67.86 ± 1.54 g/100g) and leaves (63.28 ± 2.38 g/100g) contain the highest amount. Alkaloids are a class of nitrogen containing natural compounds. Many of them are used in medicinal purpose such as vinblastine and vincristine as antitumor agents, reserpine as antihypertensive and quinine as anti-malarial. *N. indicum* has been claimed to have anti-tumor activity from hundreds of years and also been tested successfully for anti-cancer activity (Pathak S et al., 2000). The high percentage of presence of alkaloids, perhaps, supports

Table 1: Comparison of presence of various phytochemicals in leaf, stem, root and whole plant of *N. indicum*

Chemicals	Presence of phytochemicals[b]			
	Leaves	Stem	Root	Whole plant
Tannin	+	+	+	+
Phlobatannin	+	+	+	+
Cholesterol	+	-	-	-
Terpenoid	+	+	+	+
Glycoside	+	+	+	+
Phenolics	+	+	+	+
Flavonoid	+	+	+	+
Steroid	-	+	-	-
Anthraquinone	-	-	+	-
Saponin	+	+	+	+
Carbohydrate	+	+	+	+
Protein	+	+	+	+
Alkaloid	+	+	+	+

[b]All values are the mean of three replicates with the standard deviation.

Table 2: Comparison of the quantity of various phytochemicals in leaf, stem, root and whole plant of *N. indicum*

Chemicals	Quantitative value[c]			
	Leaves	Stem	Root	Whole plant
Alkaloid[d]	63.28 ± 2.38	61.74 ± 2.98	67.86 ± 1.54	64.44 ± 1.13
Saponin[d]	12.56 ± 0.67	9.06 ± 0.75	12.4 ± 0.45	10.57 ± 0.37
Flavonoid[e]	8.05 ± 0.19	7.48 ± 0.87	8.11 ± 0.88	7.77 ± 0.61
Total phenol[e]	69.86 ± 2.32	76.31 ± 1.63	82.53 ± 2.41	74.70 ± 3.39
Protein[e]	43.76 ± 1.60	31.66 ± 0.98	41.51 ± 1.16	39.21 ± 0.65
Lipid[e]	25.61 ± 0.07	14.88 ± 0.05	9.76 ± 0.05	12.51 ± 0.07
Soluble sugar[e]	3.59 ± 0.39	3.24 ± 0.14	1.71 ± 0.19	3.29 ± 0.37
Riboflavin[f]	0.42 ± 0.04	0.29 ± 0.04	0.35 ± 0.02	0.39 ± 0.01
Thiamine[f]	0.48 ± 0.05	0.43 ± 0.06	0.40 ± 0.06	0.44 ± 0.08
Ascorbic acid[f]	0.88 ± 0.02	0.49 ± 0.05	1.01 ± 0.06	0.7 ± 0.05
Tannin[f]	22.53 ± 0.86	16.65 ± 0.87	18.12 ± 0.44	18.06 ± 0.34
Moisture[g]	62.23 ± 2.07	54.76 ± 4.36	59.94 ± 3.14	61.64 ± 3.04
Ash[g]	2.98 ± 0.03	2.53 ± 0.0	2.78 ± 0.01	2.89 ± 0.02

[c] All values are the mean of three replicates with the standard deviation; [d] Units are in g/100g; [e] Units are in mg/g; f Units are in mg/100g; [g] Units are in %

these findings (Abe F and Yamauchi K, 1992; Valnet J. 1976).

Saponin belongs to a class of glycosides and its property includes hemolytic activity and cholesterol binding property. Saponins inhibit microbial proliferation. There are evidences of usage of saponins in traditional medicine preparation (Asl MN and Hosseinzadeh H, 2008; Xu *R*. 1996). Sapinins are natural anticancer agents (Rao AV and Sung MK, 1995)**.** Various cancer cells possess cholesterol rich plasma membrane to which saponin reacts and arrest their proliferation. In *N. indicum,* very high amount of saponin content has been found in the underground part. Overall saponin content in the whole plant is high (10.57 ± 0.37 g/100 g). The leaf, stem and root have been estimated to contain 12.56 ± 0.67 g/100 g, 9.06 ± 0.75 g/100 g and 12.4 ± 0.45 g/100 g of saponin, respectively. The high level of saponin directly correlates with the fact that *N. indicum* has been used traditionally as medicine for cancers (Abe F and Yamauchi K, 1992).

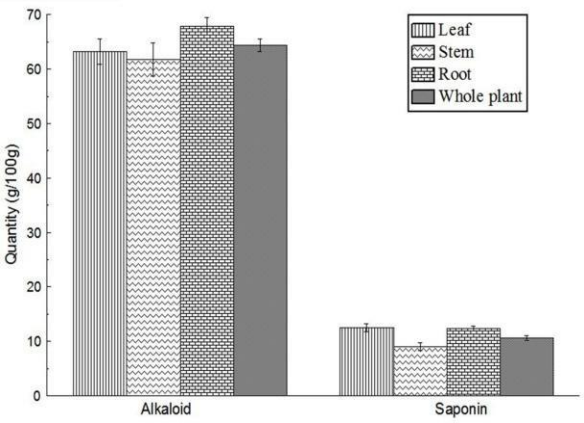

Figure 1: Quantification of alkaloid and saponin content in leaf, stem, root and whole plant of *N. indicum*.

The value represented as mean ± SD (n = 3).

Flavanoids, containing a benzopyrone, are polyphenolic plant secondary metabolites which functions primarily as antioxidants and also have cardio protective role (Hertog MG et al. 1993). The mechanism of action of flavanoids is through scavenging or chelation (Cook NC and Samman S, 1996). Flavonids suppress the progression of cancer by inhibiting estrogen producing enzymes. For example, estrogen synthesase which couples estrogen to its receptor is inhibited by flavanoids (Okwu DE and Omodamiro OD, 2005). The percentage of total flavanoids estimated in leaf, stem and root were 8.05 ± 0.19 mg/g, 7.48 ± 0.87 mg/g and 8.11 ± 0.88 mg/g respectively and the total plant had a yield of 7.77 ± 0.61 mg/g. This high level of the presence of flavonoid could be attributed to its high antioxidant capacity.

Very high quantity of total phenolic compound has been detected in all the parts of *N. indicum*. The green stem contains a cream-colored, sticky, viscous, resinous sap which is traditionally used as anti-inflammatory agent. Natural phenolics are present in all plants in varying concentrations which attribute hugely to their medicinal values. They are potent vasodilators (Padilla E et al. 2005) and are active antioxidants having hydroxyl groups possessing potent scavenging activity (Dimitrios B. 2006). The whole plant showed considerably high amount of phenolic content (74.70 ± 3.39 mg/g). The root contain highest amount (82.53 ± 2.41 mg/g) followed by stem (76.31 ± 1.63 mg/g) and leaf (69.86 ± 2.32 mg/g). Polyphenol mixtures from plant source comprises of a mixture of diverse group of phytochemicals (Wong CK et al. 1994). Identification and selective isolation of these phenolics in the future, may contribute in the field of herbal remedy as potent antioxidants.

The whole plant body possesses very high quantity of protein (39.21± 0.65 mg/g) which is preceded by the leaf having the highest amount (43.76± 1.60 mg/g). If the plant can be processed by removing or degrading certain non-nutritional factors like phytates which create complication, then it can be used as animal feed. With rich in protein, *N. indicum* showed to possess high quantity of lipid substances which adds to its energy content. Leaf of *N. indicum* contains the highest amount of lipid (25.61 ± 0.07 mg/g) compared to the other parts. Worldwide there is demand of energy fuels; so mass cultivation of *N. indicum* and other similar plants bearing high calorific value can serve as an alternative source of energy in rural areas. Table 2 shows the high content of soluble sugars by leaf and stem as 3.59 ± 0.39 mg/g and 3.24 ±0.14 mg/g respectively. There are reports of the presence of many polysaccharides isolated and purified from Chinese medicinal herbs which have turned to be bioactive, immunomodulatory (Jayabalan M et al. 1994).

Vitamin B_2 or riboflavin has anti-jaundice, anti-migraine and pain relieving effect. Riboflavin in combination with ultraviolet ray has been proved to kill harmful pathogens found in blood which cause disease (Raymond *et al*., 2006). *N. indicum* contain satisfactory amount of riboflavin. Leaf possesses the highest quantity (0.42 ± 0.04 mg/100g). There is also a high amount of thiamine estimated in *N. indicum*. The leaf contains the highest amount (0.48 ± 0.05 mg/100g) followed by stem (0.43 ± 0.06 mg/100g) and root (0.40 ± 0.06 mg/100g). Ascorbic acid or vitamin C is a potent antioxidant and terminate the chain radical reactions by electron transfer and scavenges free radicals. *N. indicum* extracts may prove to be a good antioxidant due to the presence of high concentration of ascorbic acid. The roots contain highest amount of ascorbic acid (1.01 ± 0.06 mg/100g) followed by the leaves (0.88 ± 0.02 mg/100g). The extract of the leaves proved to be a potent iron chelator and scavenger of hydroxyl radicals, superoxide anions and singlet oxygen. Sodium, calcium and potassium, salts of ascorbic acid are commercially used as antioxidant food supplements. Being water soluble, ascorbic acid is unable to protect lipid from oxidation. So the fat-soluble esters of ascorbic acid coupled with palmitate and stearate may be used as additional antioxidant supplements.

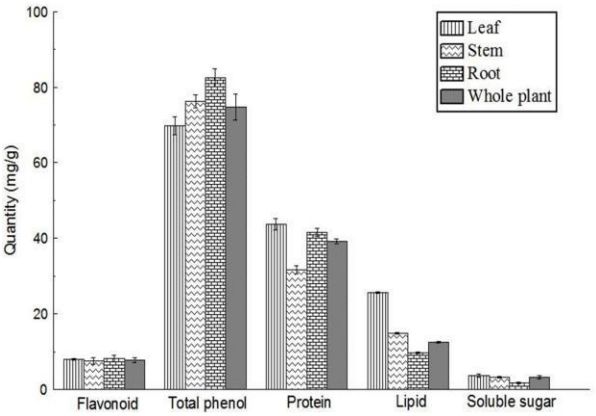

Figure 2: Quantification of flavonoid, total phenol, protein, lipid and soluble sugar content in leaf, stem, root and whole plant of *N. indicum*.

The value represented as mean ± SD (n=3).

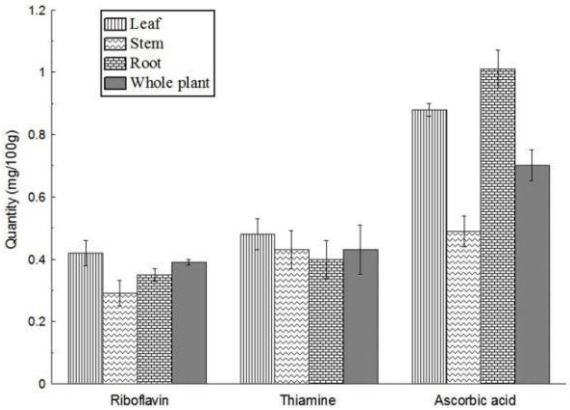

Figure 3: Quantification of riboflavin, thiamine and ascorbic acid content in leaf, stem, root and whole plant of *N. indicum*.

The value represented as mean ± SD (n = 3).

Pharmacologically, tannin is associated with antiviral activity. Various enteric viruses, polio virus and herpes simplex virus have been found to get inactivated in presence of high level of tannin (Bajaj *et al*., 1988). The leaf contain higher amount of tannin (22.53 ± 0.86 mg/100g) compared to stem (16.65 ± 0.87 mg/100g) and root (18.12 ± 0.44 mg/100g). *N. indicum* is known for its antimicrobial properties also.

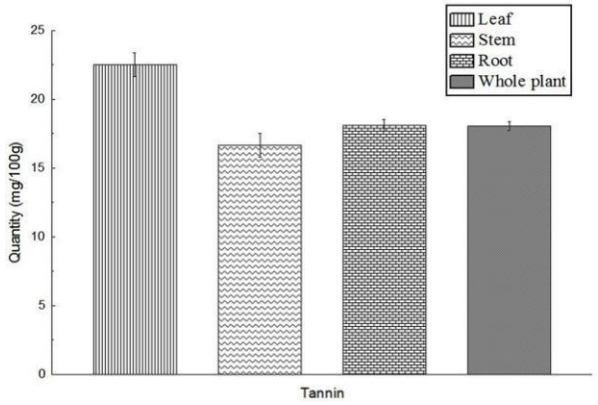

Figure 4: Quantification of tannin content in leaf, stem, root and whole plant of *N. indicum*.

The value represented as mean ± SD (n = 3).

When organic matter is burnt at very high temperature (>550) in presence of an oxidizing agent, usually oxygen, the end product is ash. In this process the organic matter loses its carbon moiety and the residue contains remaining salts and oxides, containing inorganic ions and halides. This is a test for quantification of total amount of minerals in an organic matter. *N. indicum* leaves, stem and root have been found to contain 2.98 ± 0.03 %, 2.53 % and 2.78 ± 0.01 % inorganic matter respectively. The whole plant has been found to contain 61.64 ± 3.04 % of moisture. Amongst the three major parts, the leaves have the highest quantity of moisture (62.23 ± 2.07%).

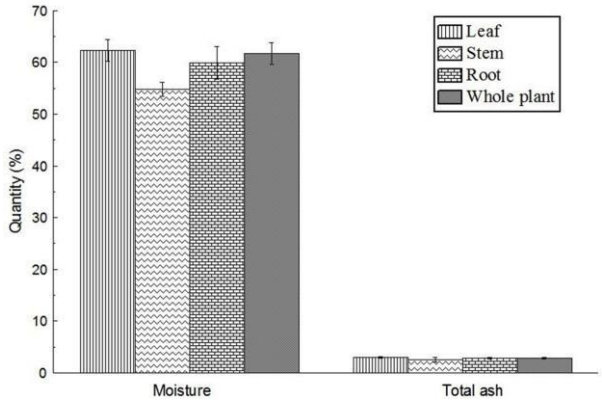

Figure 5: Quantification of moisture and ash content in leaf, stem, root and whole plant of *N. indicum*.

The value represented as mean ± SD (n = 3).

CONCLUSION

Among three major parts, the leaf and root showed the presence of highest quantity of phytochemicals; but overall, the entire plant proves to contain very high amount of phytochemicals. Many of them possess strong bioactive property. This may be used as extra-nutritional supplement in small quantities for proper health. *N. indicum* is generally considered as a poisonous plant mainly due to the presence of cardiac glycosides such as oleandrin, oleangdrigen, folineriin, adynerin and digitoxigenin; but very little case of *Nerium* lethal poisoning has been documented. Toxic Exposure Surveillance System (TESS) has reported that in 2002 there were 874 human exposures to *Nerium*. In spite of this high exposure level, only 3 lethal cases to *Nerium* poisoning has been reported (Goodrich RP et al. 2006). Augustus GDPS (2002) and his collaborators showed how bio-induction studies could prove to be useful to improve the yield of plant based chemicals**.** Similar approach is warranted towards *N. indicum*. Specifically identification and modification of the genes responsible for the desired phytochemical trait could help to maximize their yield. Though *N. indicum* is used as traditional medicine but it is advised not to intake any part of the plant. Rather approach can be taken to stimulate the production of these phytonutrients and phytochemical products to be used as alternative medicine. Our future approach is to identify and isolate different phytochemicals from *N. indicum* and test these chemicals for their antioxidant, immunomodulatory and anti-cancer properties.

ACKNOWLEDGEMENTS

The authors would like to thank Mr. Avinash Chaudhuri for his contribution towards collection of plant material. The authors are also grateful to Mr. Manoj Lama, Mr. Pokhraj Guha, Mr. Somit Dutta, Mr. Suman Tamang and Mr. Manas Ranjan Saha providing their expert views and guidance throughout the experients.

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