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QUANTITATIVE AND QUALITATIVE PHYTOCHEMICAL ANALYSIS IN BARK OF *NOTHOPEGIA RACEMOSA* (DALZ.) RAMAM. COLLECTED FROM GADIKALLU, SHIMOGGA, KARNATAKA

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ABSTRACT: Plants are a source of bioactive compounds produced by different parts of the plant, maybe bark, leaves, flowers, roots, fruits, and seeds, during metabolic activities. These secondary metabolites may have protective and disease preventive properties, hence used by Traditional practioner to treat various ailments. Most plants of the Western Ghats of India remain unknown, poorly understood or unestablished for their effects on health because of unexplored phytochemical studies or insufficient scientific data. Improvement in health is possible through the intake of phytochemical supplements, which reduce oxidative stress. In recent years research on phytochemicals and search for novel high quality, inexpensive source of medicine is increasing as these do not have harmful side effects. In the present study *Nothopegia racemosa*, a member of Anacardiaceae, is investigated for the presence of phytochemical compounds, which would lead to further pharmacological studies. The investigation was carried out using dried and powdered bark extract using water, hydro alcohol, chloroform, petroleum ether, and hexane as solvents. The qualitative analysis revealed the presence of tannins, alkaloids, saponins, glycosides, terpenoids, flavonoids, mucilage, steroids, and phenols. Quantitative analysis showed the presence of a significant concentration of phytochemicals.

INTRODUCTION: Fauna and Flora are a source of medicine since ancient times. Among these floras are utilized by Tribal and Traditional practioner for curing many diseases. In recent times phytochemicals are useful in formulating many drugs in the pharmaceutical industries. Local communities and folk healers use different parts of plants such as root, stem, flowers and fruits for treating various ailments.

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Western Ghats of India is enriched with diversified vegetation; the phytogeographical regions is about 1500 km that extends in the South West coast of India covering the states like Goa, Maharashtra, Karnataka, Tamil Nadu, and Kerala,[1] most plants of Western Ghats are rich in phytochemicals which are poorly understood and without sufficient scientific data. Search for novel high quality, inexpensive sources of medicine has remained a major concern in developing countries.

Sharavathi Wildlife Sanctuary in the Western Ghats harbours rich tree diversity with 100 species belonging to 40 families in 1.2 hectare. To the floristic richness of Anacardiaceae, members contribute to the frequency of 0.77, Density 2.90, and Abundance of 23.15. It is also one of the mega

center of many endemic species. *Nothopegia racemosa* being endemic to this habitat constitutes to the Frequency of 0.33, the density of 0.73, an abundance of 2.20, and species richness value of 3.80 [2].

N. racemosa is an under tree belonging to the family Anacardiaceae, with characteristic black exudates from the bark. It is commonly known as Dalzell's Nothopegia, Amberi in Marathi, Kadukudi in Kannada. It is endemic to India and Srilanka. In India, it is distributed in the Western Ghats, South and Central Sahyadris.

Phytosociological studies of medicinal plants reveals that *N. racemosa* is traditionally used as antimicrobial, anti-diabetic, antioxidant, anticancer and anti-inflammatory agent in curing different ailments.[3] Based on the available data much research on phytochemical and medicinal properties has not been conducted in *N. racemosa*. Hence the present investigation was carried out to quantify the phytochemicals and to explore its therapeutic properties.

REVIEW OF LITERATURE: The distribution of Anacardiaceae members is studied by different researchers. The structural diversity of Charmudy reserve was evaluated, and it found that the ground vegetation is dominated by *N.racemosa* along with other genera. *N. racemosa* plant species has about 12.3 Family Importance Value (FIV) index in Charmudy reserve forest, account for the 9 percent of total abundance of species in the selected area.[4.] Distribution of wild edible fruit trees of Anacardiaceae was found that *N. castaneifolia*, commonly called as Amberi is used for pickling and also eaten raw [5]. Ethno floristic diversity and distribution of medicinal tree species reveals that *N. racemosa* is found to be endemic to India with an abundance of 834 and 6 stems/60 ha. [6]

A survey conducted in the central Western Ghats reveals that, *N. racemosa* is distributed abundantly along with other medicinal plants.[7] Bharathi *et al.* worked on the Diversity and regeneration status of tree species in the sacred groves of central Western Ghats, and they revealed that *N. racemosa* and *N. beddomei* are most abundant tree species in the study area with the density per hectare was found to be 13.54 and 10.41 respectively [8].

Work on the diversity of woody medicinal plants of Megamalai wildlife sanctuary shows that *N. racemosa* is abundant [9]. Singh worked on the endemic angiosperms and reported that work on the ethno floristic diversity in central Western Ghats and found *N. castaneifolia* leaves are used as antiseptic [10]. *N. beddomei* and *N. racemosa* were strictly endemic to Western Ghats [11].

MATERIALS AND METHODS:

Plant Material Collection: Bark of *N. racemosa* was collected on 16th June 2019 from their natural habitat Western Ghats region in Thattapura forest, Gadikallu, Thirthalli taluk, Shimoga District, Karnataka, India. The plant was identified by taxonomist and deposited at of Department of Studies and Research in Botany, Tumkur University Tumakuru. The identification number is TUBNR-2018.

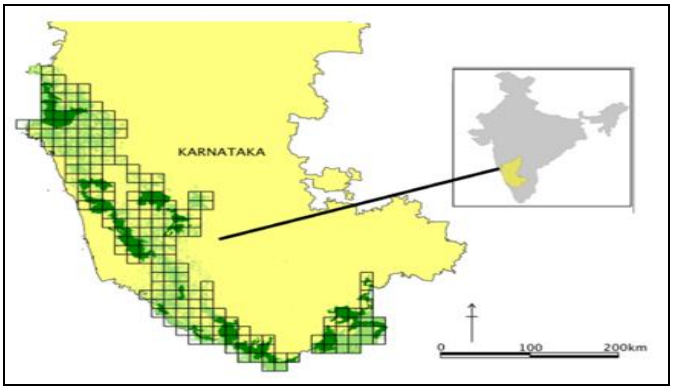

FIG. 1: STUDY AREA WHERE *N. RACEMOSA* PLANT SAMPLES WERE COLLECTED

Cleaning and Drying: The collected Bark was chopped into small pieces, cleaned to get rid of dust, and air-dried at room temperature for 21 days to remove moisture content. The dried plant material was ground into powder and preserved in air-tight containers at 4°C in the refrigerator for further analysis.

Chemicals: The chemicals used in the present study were of analytical grade.

Crude Extraction: The powdered plant material was subjected to solvent extraction for the separation of bioactive compounds using selective solvents such as Hexane, Petroleum ether, Chloroform, Hydro-alcohol and water through standard extraction procedures in the laboratory in August 2019.

Qualitative Phytochemical Analysis: Screening of phytochemicals was carried out by standard methods [12, 13].

Test for Alkaloids:
Dragendoff's Test: 0.2ml of the sample was taken in a test tube, add 0.2ml of HCl and 2-3 drops of Dragendoff's reagent, the appearance of orange or red precipitate and turbid solution indicates the presence of alkaloids.

Test for Carbohydrates:
Molisch's Test: 0.2 ml of sample was mixed with few drops of Molisch's reagent (α- napthol dissolved in alcohol), and 0.2 ml of sulphuric acid was added along the sides of the test tube, the appearance of a purple coloured ring indicates a positive test.

Test for Tannins: Braymer's test: 0.2 ml of plant extract was mixed with 2 ml of water and heated on the water bath for 10 minutes. The mixture was filtered, and ferric chloride was added to the filtrate and observed for a dark green solution, which indicates the presence of tannin.

Test for Terpenoids:
Salkowki's Test: Take 0.2 ml of Bark sample extract in a test tube and 0.2 ml of chloroform. Carefully add Concentrated H_2SO_4.

The appearance of the Reddish-brown layer at the interface confirms the presence of Terpenoids.

Test for Glycosides: 0.2 ml of sample was mixed with 0.2 ml of chloroform; add 0.2ml of acetic acid, and the mixture was cooled on ice. Sulphuric acid was added carefully; colour change from violet to blue to green indicates the presence of glycosides.

Test for Steroids:
Lieberman Burchardt Test: 0.2 ml of sample was mixed with 0.2 ml of chloroform. To this, 0.2 ml of concentrated sulphuric acid was added. The appearance of red colour in the lower layer indicates the presence of steroids.

Test for Saponins:
Saponin Test:
Foam test: Add 0.6 ml of water to 0.2ml of bark sample extract in a test tube and shake vigorously. The appearance of persistent Foam confirms the presence of Saponin.

Test for Protein: Add 0.1 ml of sample and 0.1 ml of H_2SO_4, red ring formation confirm the presence of Protein.

Test for Flavonoids:
Alkaline Reagent Test: 0.2 ml of plant extract was taken in a test tube and mixed with dilute sodium hydroxide solution. To this, dilute hydrochloric acid was added. The yellow solution t turns colourless indicates the presence of flavonoids.

Test for Phenol: Ferric chloride test: To 0.2 ml of extract add 0.4 ml of distilled water and few drops of 10% aqueous ferric chloride solution, formation of blue or green colour indicate the presence of phenols.

Mucilage Test: Take 0.2 ml of Bark sample extract in at test tube, add 0.2 ml of absolute alcohol and allow it to dry. The precipitation occurs, confirms the presence of mucilage.

Volatile Oil: 0.2 ml of extract was treated with few drops of dilute hydrochloric acid. The appearance of a white precipitate indicates the presence of volatile oils.

Quantitative Analysis of Phytochemicals: Quantitative phytochemical analysis is carried out for total alkaloids, total flavonoids, total phenol, total saponins, total steroid and total tannin content in different solvent extracts of leaf and bark of *N. racemosa.*

Determination of Total Alkaloid Contents: Total alkaloid content of bark of *N. racemosa* were analyzed by standard methods [14, 15]. To 1ml of test, extract add 5 ml of phosphate buffer (pH 4.7). Add 4ml of chloroform to 5ml of Bromocresol green and shake well. Make up the solution with chloroform in a 10ml volumetric flask.

Absorbance at 470 nm was recorded against the reagent blank. A standard calibration plot was generated at 470 nm using Spectra Max i3X for standard Capsaicin.

Determination of Total Flavonoid Contents: Total flavonoid content of Leaf and Bark were analyzed by standard methods [16].

100 µl of sample add 300 µl of methanol, 100 µl of Aluminum chloride, 100 µl of Sodium Potassium

tartarate, and 300 µl of distilled water. Shake the test solution vigorously. After 30 min of incubation, absorbance at 415 nm was recorded. Using quercetin, standard calibration was generated at 415nm. At room temperature, the mixture was incubated for 5 min. The concentrations of flavonoid in the test sample were calculated from the calibration point and expressed as mg of Quercetin Equivalent per gram of sample.

Determination of Total Phenol Contents: Total Phenolic content in the bark was analyzed by standard methods [14]. To 100 µl of sample, add 900 µl of distilled water, 150 µl of Folin-Ciocalteau reagent. For 5 minutes at room temperature, incubate the mixture. Then add 400 µl of 20% Na_2Co_3. The mixture was agitated and left to stand for 30 min at room temperature. The absorbance of the standard was measured at 765 nm using blank. A standard curve of absorbance against gallic acid concentration was prepared and used for estimation of total phenols content in the test sample. The concentrations of phenols in the test sample were calculated from the calibration point and expressed as mg gallic acid equivalent per gram of sample.

Determination of Total Saponin Contents: Total Saponin content in the bark was analyzed by standard methods [14]. Dissolve the test extract in 80% methanol. Add 2 ml of vanillin in ethanol, 2 ml of 72% sulphuric acid solution, and mix well. Heat the test solution in a water bath for 10 minutes at 60°C with occasional shaking. Absorbance at 544 nm was recorded against the reagent blank. A standard calibration plot was generated at 544 nm using known standard Diosgenin. The concentrations of Saponin in the test sample were calculated from the calibration point and expressed Diosgenin mg equivalent per gram of sample.

Determination of Total Steroid Contents: Total Steroid content in the bark was analyzed by standard methods [14].

1ml of the test sample was dissolved with their respective solvent; add 2 ml sulphuric acid (4N), 2 ml of ferric chloride (0.5% w/v), and 0.5 ml of Potassium ferric cyanide solution (0.5% w/v). Heat the test solution for 30 minutes in a water bath maintained at 70±2°C with occasional shaking.

Absorbance at 780 nm was recorded against the reagent blank. A standard calibration plot was generated at 780 nm using known β- Stigmasterol. The concentrations of steroids in the test sample were calculated from the calibration point and expressed β- Stigmasterol mg equivalent per gram of sample.

Determination of Tannin: Total Tannin content in the bark was analyzed by standard methods [16].

To 100 µl of a sample, add 7.5 ml of water, 500 µl of Folin-Denis reagent, and 1000 µl of 35% sodium carbonate and make up the solution up to 10 ml.

After 30 minutes of incubation, absorbance was recorded at 725nm. A standard calibration plot was generated at 725 nm using standard Gallic acid. The concentration of tannin in the test sample was calculated from the calibration point and expressed mg tannic acid equivalent per gram of sample.

RESULTS AND DISCUSSION:

Qualitative Analysis: The Qualitative phytochemical analysis of bark extract of *Nothopegia racemosa* was carried out using five different solvents like Hydro alcohol, Hexane, Petroleum ether, Water, and Chloroform.

Even though all extracts showed the presence of different phytochemicals, the presence of alkaloid, terpenoid, phenol, carbohydrate, and protein was answered by all the extracts. The presence of volatile oil was not observed by any of the extracts, and the presence of tannin was seen only in the water extract. **Table 1** explains the presence or absence of phytochemicals.

TABLE 1: QUALITATIVE PHYTOCHEMICAL ANALYSIS OF *N. RACEMOSA* BARK EXTRACTS

Types of Tests	Hydro-alcohol	Hexane	Pet-ether	Water	Chloroform
Alkaloid Dragendoff's Test	+	+	+	+	+
Carbohydrate Molisch's Test	+	+	+	+	+
Tannin	-	-	-	+	-
Terpenoid Salkowki's Test	+	+	+	+	+
Glycoside	+	-	-	+	-
Steroid Lieberman Burchardt Tests	+	-	-	+	-
Saponin Foam Test	+	-	+	+	+

Flavanoid Alkaline Reagent Test	+	-	+	+	+
Mucilage Test	-	+	+	-	-
Volatile Oil	-	-	-	-	-
Protein	+	+	+	+	+
Phenol	+	+	+	+	+

+: Present, -: Absent

Quantitative Phytochemical Analysis: Total content of bioactive compounds like alkaloids, tannin, terpenoid, steroid, Saponins, and Flavonoids are quantitatively analyzed, evaluated, and represented by using standard graphs.

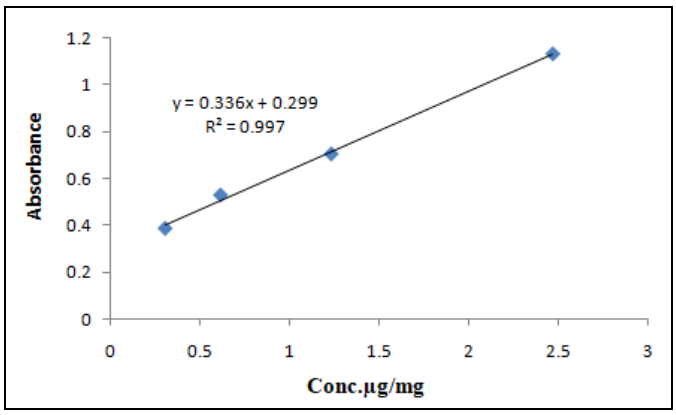

FIG. 2: STANDARD GRAPH OF CAPSAICIN

TABLE 2: ALKALOID CONTENT IN THE BARK EXTRACT OF *N. RACEMOSA*

Sample	Conc. (ug/ml)	Abs	Conc. Alkaloid (µg CE)	Conc. Alkaloid (µgCE/mg)
Hydro alcohol Extract	300	0.456	0.47	1.56
Hexane Extract		0.422	0.37	1.22
Chloroform Extract		0.386	0.26	0.86
Pet. Ether Extract		0.375	0.23	0.75
Water Extract		0.419	0.36	1.19

Alkaloids in *N. racemosa* bark extracts showed significant results by plotting standard curve generated at 470 nm using standard capsaicin, Hydro-alcohol extract (1.56 µgCE/mg) showed the highest alkaloids concentration followed by water (1.19µgCE/mg), hexane (1.22µgCE/mg), chloroform (0.86 µg CE/mg) and least in petroleum ether extract (0.75 µg CE/mg) **Fig. 2**, **Table 2**.

Flavonoids: Flavonoids in *N. racemosa* bark extracts showed significant results by plotting standard curve generated at 415 nm using standard quercetin, Hydro-alcohol extract (682.50 µg QE/mg) showed the highest flavonoid concentration followed by water (617.50 µg QE/mg), petroleum ether (421.25 µg QE/mg), chloroform (367.50 µg QE/mg) and least in hexane extract (363.75 µg QE/mg) **Fig. 3**, **Table 3**.

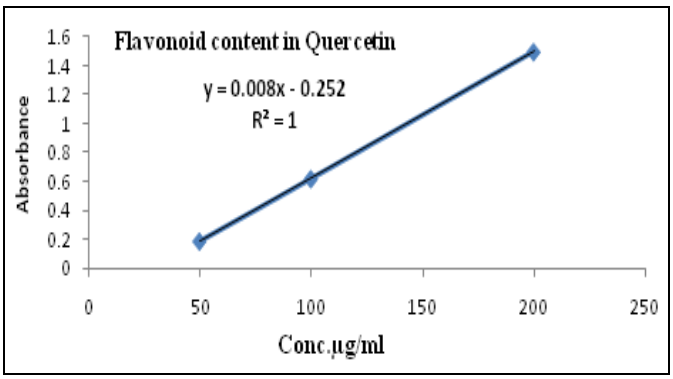

FIG. 3: FLAVONOID CONTENT OF QUERCETIN

TABLE 3: FLAVONOID CONTENT IN THE BARK EXTRACT OF *N. RACEMOSA*

Sample	Conc. (ug/ml)	Abs	Conc. Flavonoids (µg CE)	Conc. Flavonoids (µgCE/mg)
Hydro alcohol Extract	100	0.294	68.25	682.50
Hexane Extract		0.039	36.38	363.75
Chloroform Extract		0.042	36.75	367.50
Pet. Ether Extract		0.085	42.13	421.25
Water Extract		0.242	61.75	617.50

Phenol Content: Phenol in *N. racemosa* bark extracts showed significant results by plotting standard curve generated at 765 nm using a standard Gallic acid in that, Hydro-alcohol (99.31 µg GAE/ml) showed the highest phenol concentration followed by water (92.76 µg GAE/ml), petroleum ether (71.72 µg GAE/ml), hexane (68.62 µg GAE/ml) and least in chloroform (45.52 µg GAE/ml) **Fig. 4**, **Table 4**.

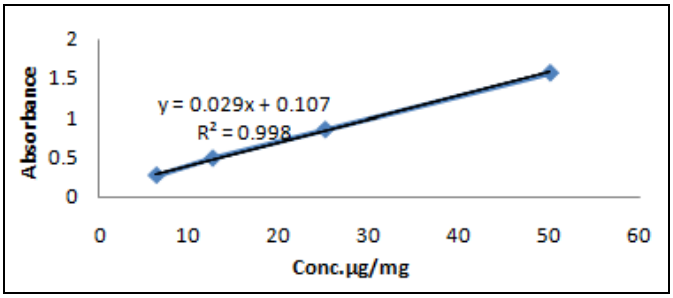

FIG. 4: STANDARD GRAPH OF GALLIC ACID

TABLE 4: PHENOLIC CONTENT IN THE BARK EXTRACTS *N. RACEMOSA*

Sample	Conc. (ug/ml)	Abs	Conc. Phenol (µg CE)	Conc. Phenol (µgCE/mg)
Hydro alcohol Extract	100	0.395	9.93	99.31
Hexane Extract		0.306	6.86	68.62
Chloroform Extract		0.239	4.55	45.52
Pet. Ether Extract		0.315	7.17	71.72
Water Extract		0.376	9.28	92.76

Saponin: A standard calibration plot was generated at 544 nm using standard Diosgenin. The concentrations of saponins in the test sample were calculated from the calibration point and expressed Diosgenin mg equivalent per gram of sample. Saponin in *N. racemosa bark* extracts showed significant results, Hydro-alcohol extract (0.53 mg DE/ml) showed highest saponin concentration followed by water (0.47 mg DE/ml), petroleum ether (0.43 mg DE/ml), chloroform (0.36 mg DE/ml) and least in hexane extract (0.34 mg DE/ml) **Fig. 5**, **Table 5**.

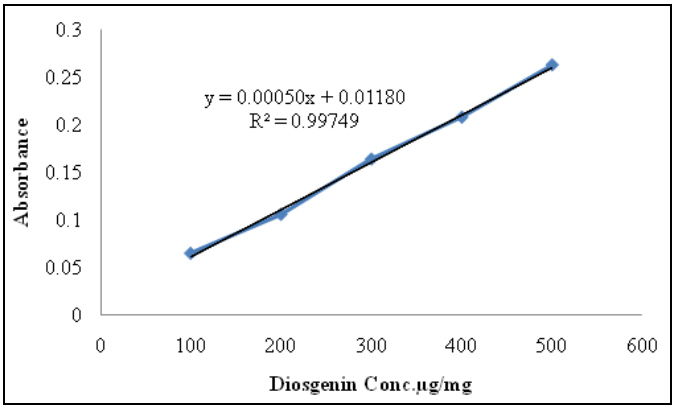

FIG. 5: STANDARD GRAPH OF DIOSGENIN

TABLE 5: SAPONIN CONTENT IN THE BARK EXTRACT OF *N. RACEMOSA*

Sample	Conc. (ug/ml)	Abs	Conc. Saponin (ug DE/100ul sample)	Conc. Saponin (mg DE/ml sample)
Hydro alcohol Extract	300	0.053	82.40	0.27
Hexane Extract		0.048	72.40	0.24
Chloroform Extract		0.055	86.40	0.29
Pet. Ether Extract		0.071	118.40	0.39
Water Extract		0.041	58.40	0.19

Saponin in *N. racemosa* bark extracts showed significant results by plotting standard curve generated at 544 nm using a standard Diosgenin in that, petroleum ether extract (0.39 mg DE/ml) showed the highest saponin concentration followed by chloroform (0.29 mg DE/ml), Hydro-alcohol (0.27 mg DE/ml), hexane extract (0.24 mg DE/ml) and least in water extract (0.19 mg DE/ml) **Fig. 5**, **Table 5**.

Steroid: Steroid in *N. racemosa* bark extracts showed significant results by plotting standard curve generated at 780 nm using a standard β-Stigmasterol in that, Hydro-alcohol bark extract (4.17 mg βSSE/g) showed highest steroid concentration followed by water (2.08 mg βSSE/g), hexane (0.63 mg βSSE/g), chloroform (0.35 mg βSSE/g) and least in petroleum ether extract (0.14 mg βSSE/g) **Fig. 6**, **Table 6.**

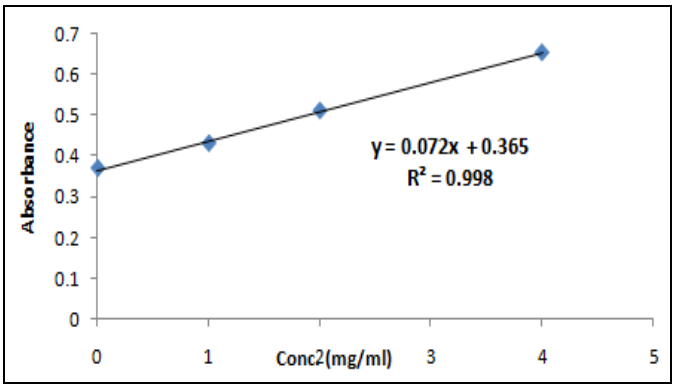

FIG. 6: STANDARD GRAPH OF STEROID

TABLE 6: STEROID CONTENT IN THE BARK EXTRACT OF *N.RACEMOSA*

Sample	Conc. (ug/ml)	Abs	Conc. Steroid (mg βSSE/200ug)	Conc. Steroid (mg βSSE/g)
Hydro alcohol Extract	200	0.425	0.83	4.17
Hexane Extract		0.374	0.13	0.63
Chloroform Extract		0.370	0.07	0.35
Pet. Ether Extract		0.367	0.03	0.14
Water Extract		0.395	0.42	2.08

Tannin: Tannin in *N. racemosa* bark extracts showed significant results by plotting standard curve generated at 725 nm using a standard Gallic acid in that, water extract (130.00 µg GAE/mg) showed the highest tannin concentration followed by, Hydro-alcohol (90.00 µg GAE/mg), hexane (73.33 µg GAE/mg), petroleum ether (63.33 µg GAE/mg) and least in chloroform extract (50.00 µg GAE/mg) **Fig. 7**, **Table 7**.

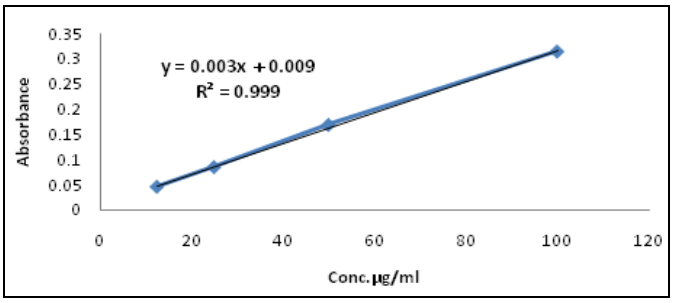

FIG. 7: STANDARD GRAPH OF GALLIC ACID

TABLE 7: TANNIN CONTENT IN THE BARK EXTRACT OF *NOTHOPEGIA RACEMOSA*

Sample	Conc. (ug/ml)	Abs	Conc. Tannin (µg GAE)	Conc. Tannin (µg GAE/mg)
Hydro alcohol Extract	100	0.036	9.00	90.00
Hexane Extract		0.031	7.33	73.33
Chloroform Extract		0.024	5.00	50.00
Pet. Ether Extract		0.028	6.33	63.33
Water Extract		0.048	13.00	130.00

The presence of alkaloid, terpenoid, phenol, carbohydrate, and protein was answered by all the extracts in *N. racemosa*. Similarly, preliminary phytochemical screening of leaf and bark in *Anacardium occidentale* using aqueous, acetone, chloroform, ethylacetate, hexane, methanol revealed alkaloids Carbohydrates; however the extracts also showed the presence of flavonoids and cardiac glycosides [17]. The leaf and bark methanolic extract of *Holingarna graham* reveals the presence of reducing sugar, alkaloids, and phenols [18]. Tapan *et al.,* from the chloroform/ methanol extract of *Sapindias pinnata* bark isolated Ergosteryl triterpenes [19].

The only aqueous bark extract of *N. racemosa* showed the presence of tannin. In *Mangifera indica*, leaf ethanol extract showed the presence of tannin along with other compounds such as alkaloids, flavonoids, steroids, and cardiac glycosides [20]. In *N. racemosa*, Hydro-alcohol showed the highest phenol concentration followed by water, petroleum ether, hexane, and least in chloroform similar results were observed in the seed extracts of *Buchanania lanzan* [21]. Presence of Proteins is answered by all extracts in *N. racemosa* similarly Hydro alcohol and Chloroform bark extract in *Scelerocarya birrea* an African Anacaridaceae member answered for proteins [22].

The total Phenolic estimation, hydroalcoholic extract of *N. racemosa* was high with 9.93 µg GAE/100µl. The leaf and bark extracts of *N. heyneana* in solvent extracts such as Aqueous, petroleum ether, chloroform and ethanol showed the presence of Phenol and further quantification of total phenol showed high content 101.6 GAE/g [23].

Phenols and flavonoids have potentiality to prevent chronic-oxidation-related diseases, such as diabetes and micro-and macro-cardiovascular diseases [24]. In *Rhus triloba*, total phenolic content was estimated by Folin-Ciocâlteu; flavonoids and anthocyanins were quantified using aluminum chloride and differential pH methods. Gallic acid, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, quercetin were used as a standard compound to calculate the concentration as an equivalent [25].

Chemical analysis in aqueous extract of *Mangifera indica* leaves revealed a high level of Phenolic compounds, Tannins, and Flavonoids in strepto-zotocin-induced diabetic rats characterizing an antidiabetic therapy [26]. The quantitative phytochemical mineral content of the leaves and stem bark of *Spondia mombin* did not show any significant difference. But revealed the presence of alkaloids, flavonoids, tannins, cyanogenic glycosides, saponins, phenols, oxalates and

phytates in a varying amount which were associated with medicinal value for centuries [27].

N. racemosa showed the presence of a variety of phytochemicals and will be tested for different pharmacological activities in further studies.

CONCLUSION: The qualitative phytochemical profile of bark extract of *N. racemosa* proves that the plant is a potential source of secondary metabolites. Quantitative estimation revealed the presence of the considerable amount of alkaloids, phenols, terpenoids, saponins, tannins, and flavonoids in different extracts. The purification of phytochemicals from the selected plant and further investigation relating to pharmacological activities may throw light on new findings, which can be used in the treatment of diseases and maintenance of human health.

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