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Preliminary phytochemical evaluation and antioxidant properties of an endemic plant *Nothopegia travancorica* Bedd. (Anacardiaceae) in Southern Western Ghats, India

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Abstract
The phytochemical evaluation of solvent extracts of leaves and stem bark of an endemic tree *Nothopegia travancorica* Bedd. ex Hook.f. (Anacardiaceae) revealed that the presence of saponin, sterols, carbohydrates, tannins, proteins and flavonoids. The study also quantified the total phenol content and flavonoids from different solvent extracts of stem bark that showed high content of phenol (106.6 µg GAE/g) and flavonoid (63.32 µg Rutin/g) in ethanol extracts respectively. Antioxidant activity of DPPH radical is scavenged by phytocompounds antioxidants through the donation of a proton forming the reduced DPPH. It showed the evidence of an endemic plant to have a rich source of metabolites for positive therapeutic properties which could be isolated and characterized for further development of safe phytomedicine.

Keywords: endemic plant, *Nothopegia travancorica*, phytochemicals, phenolics, flavonoids

Introduction

Higher plants are vital sources of enormous varieties of phytocompounds, and which could be used as therapeutic agents and medicinal drugs that are used for treating various kinds of human ailments since time immemorial. Plants are continuously exploited for their medicinal and other purposes selectively by the human community based on their experience with plants in trial and error since the origin of human race. There has been estimated about 30,000 medicinally important plants worldwide and with more than 1,75,000 different known chemical compounds of plant origin screened for various healing purposes [1]. In India, about 8000 plants are well known medicinal species either based on traditional knowledge or scientific screening, most of which are concentrated in biodiversity hotspot areas of Eastern Himalaya and Western Ghats [2]. Until now several of endemic plants are not screened for their bioactivity due to inaccessible and unavailability of common man which are inhabited in remote forests and camouflage with lush vegetation.

Quality of natural drugs can be determined by its identity, purity and content and other chemical, physical, biological properties or by the manufacturing processes. For quality determination of traditional medicine, the traditional methods are procured and studied, and document the traditional information about the identity and quality assessment are interpreted in terms of modern scientific assessment [3]. Plants drugs are easily available, less expensive, safe and efficient, which already have estimated minimal side effects. In contrast to synthetic single chemical therapeutics, phytomedicines are potentially beneficial through additive and synergetic action [4]. Though plant derived drugs should be carefully evaluated before their use and are to be determined phytochemical groups and their properties. Usually, the secondary metabolites such as alkaloids, flavonoids, phenolics, saponins, terpenoids and quinines are predominantly present in higher medicinal plants [5]. Plant drugs must be screened and characterized by appropriate scientific evaluation before therapeutic use. There are number of endemic plants still exists in the wild, perhaps either traditional or scientific knowledge not known their biological values. The present investigation is aimed to screen the phytochemical and antioxidant properties of an endemic pant *Nothopegia travancorica* for its therapeutic importance.

Materials and Methods

Collection of plant materials

The leaves and stem barks of *Nothopegia travancorica* Bedd. ex Hook.f. (Fig.1) were collected carefully from the evergreen forest areas of Thirukurungudi range of Western Ghats, Agasthiyamalai Biosphere Reserve, southern India. The collected materials were shade dried for 15 days and finally pulverised separately into a coarse powder. It was stored in a well closed container free from moisture until uses.

Fig 1: Flushing twig of *Nothopegia travancorica* Bedd. Ex Hook.f.

Extraction

The stem bark and leaf powders of 200 grams of each were extracted separately in Soxhlet's apparatus sequentially in

250 ml of Petroleum Ether, Chloroform, Ethanol and Aqueous. The process was run for 48 hrs after which the sample was concentrated using rotator evaporator. Extract yield were calculated and consistency and colour of the extracts were also noted. The dried extracts were weighed and kept in labelled sterile specimen bottles at 20^0C.

Preliminary phytochemcial screening

The qualitative phytochemical screening was performed for testing different chemical groups present in extracts by standard phytochemical analysis [6].

Tests for alkaloids: Extract (100 mg) was treated with few drops of Dragendorff's reagent [Potassium bismuth iodide solution]. Formation of orange brown precipitate indicated the presence of alkaloids. To 100 mg of extract small quantity of Wagner's reagent [Solution of iodine in potassium iodide] was added. Presence of reddish brown precipitate if alkaloids are present. To 100 mg of extract small quantity of Hager's reagent [saturated solution of Picric acid] was added. Formation of yellow precipitate indicated the presence of alkaloids.

Test for saponin: Powdered drug extract on shaking vigorously with water results into persistent foam.

Tests for sterols: 200 mg extract was boiled with 3 ml of dil. H_2S0_4 in a test tube for 5 min and filtered while hot. Cool and added the equal volume of C_6H_6 and $CHCl_3$, shake well and separated the organic solvent and added the NH3. The ammonical layer turned pink or red. Alcoholic extract was treated with 1 ml pyridine and 1 ml of sodium nitroprusside. Pink to red colour appears. 1ml of test extract in a test tube then add 5 ml of anhydrous acetic acid and shake well. Take 4 drops of the above mixture and place in a porcelain dish, and then add one drop of conc. H_2SO_4. A change of colour from rose, through red, violet and blue to green (Lieberman-Burchard test).

Tests for cardiac glycosides: Extract (2 ml) was treated with 0.4 ml of glacial acetic acid containing a trace amount of $FeCl_3$ and 0.5 ml of concentrated H_2S0_4 was also added by the side of the test tube (Keller-Killians test). Persistent blue color appeared in the acetic acid layer if cardiac glycosides were present.

Test for tannins: To 5 ml of extract few drops of 5% $FeCl_3$ was added. Presence of deep blue black colour indicated the presence of tannins [7].

Tests for proteins: A little extract was taken with 2 ml of water and 0.5 ml of concentrated HNO_3 was added to it. Yellow colour is obtained if proteins are present. To 5 ml of extract 4% NaOH was added along with few drops of 5% $CuSO_4$ solution. Violet or pink colour appeared indicated the presence of proteins.

Tests for terpenoids: Extract (5 ml) was treated with 5 ml $CHCl_3$ with few drops of conc. $H2SO_4$, shake well and allowed to stand for some time. Formation of yellow coloured lower layer indicated the presence of triterpenoids. Extract (5 ml) was treated with few drops of acetic anhydride, boiled and cooled, conc. H_2SO_4 was added from the sides of the test tube showed a brown ring at the junction of two layers and the upper layer turns green which showed the presence of Steroids and formation of deep red colour indicated the presence of triterpenoids.

Tests for carbohydrates: In a test tube containing 5 ml of extract, few drops of freshly prepared 10% alcoholic solution of α- naphthol was added and shaken/stirred for few min. Then 5 ml of conc. H_2SO_4 was added from sides of the test tube. Violet ring was formed at the junction of two liquids, indicated the presence of carbohydrates.

Test for fixed oils and fats: Small quantity of extract was pressed the between two filter papers, the stain on I filter paper indicated the presence of fixed oils. The extract was evaporated to get 10 ml of extract. To the extract 25 ml of 10% NaOH was added, then it was boiled in water bath for 30 min. The extract was cooled and excess of sodium sulphate was added. Soap was formed at the top and filtered. To the filtrate H_2SO_4 was added which was evaporated. The extract was dissolved in ethanol and few drops of $CuSO_4$ and NaOH was added. Clear blue solution indicated the presence of fats.

Tests for flavonoids: When 5 ml of extract was treated with few drops of 5% lead acetate solution, white precipitates appeared. To 5 ml of extract 5 ml of 95% ethanol was added along with dilute HCl from sides of test tube. Few fragments (0.5 g) of magnesium turnings were also added. Presence of slight pink colour indicated the presence of flavonoids. To 5 ml of extract few drops of NaOH solution was added. Formation of an intense yellow colour, which turns to colourless on addition of few drops of dil. H_2SO_4 indicated the presence of flavonoids.

Determination of total phenols

The total phenol content was determined by the Folin Ciocalteu method [8] for ethanol extract of stem bark of *Nothopegia travancorica*. Different concentrations of the extracts were taken to that 0.1 ml of Folin Ciocalteu reagent and 2.5 ml of 0.2 N Na_2CO_3 were added and the mixture incubated for 30 min at room temperature. Distilled water was used as blank. Absorbance was measured at 760 nm using spectrophotometer. Gallic acid was used as standard and the results were expressed as µg of gallic acid equivalents per gram dry mass of extract (µg GAE/g) [9].

Estimation of total flavonoid content

The total flavonoid content was determined by the aluminium chloride colorimetric assay. In a test tube, 0.3 ml of extracts, 3.4 ml of 30% methanol, 0.15 ml of $NaNO_2$ (0.5 M) and 0.15 ml of AlCl3.6H2O(0.3M) were mixed. 1 ml of NaOH was added after 5 min. The absorbance was taken at 506 nm against the blank. The standard curve with the reference of Rutin standard solution was made. The total flavonoid content was expressed with the Rutin equivalents per g of dried fraction [10].

Determination of antioxidant activity

DPPH radical scavenging assay 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was measured using the standard protocol with slight modifications. The standard curve was made using ascorbic acid with absorbance read at 517 nm. The DPPH radical scavenging capacity was expressed in terms of Ascorbic

Acid Equivalent, as the percentage of inhibition of the assay was calculated by the following formula [11]. % Inhibition of DPPH= OD of control-OD of sample/ OD of control*100

Statistical analysis

The experimental results were performed in triplicate and expressed as the average ± standard deviations. The magnitude of the means, standard curve, standard errors, standard deviations, one way ANOVA was calculated by using Microsoft Excel 2010 Software. $P<0.05$ is accepted as statistical significance.

Results and Discussion

From leaves and stem bark of *N. travancorica* yielded considerable amount of extracts and extracts yield in all four kinds of solvents such as petroleum ether, chloroform, ethanol and aqueous (Table 1). Among the solvents ethanol showed maximum percentage of extract yields. Leaves and stem bark extracts of *N. travancorica* contained a significant amount of phytochemicals such as saponins, sterols, carbohydrates, tannins, proteins and flavonoids (Table 2). However, alkaloids, anthraquinones and cardiac glycosides are not detected in qualitative tests. Tannins and flavonoids are strongly present in all four kinds of extracts from both leaves and stem barks. The similar results were observed from *Nothopegia heyneana* [12] and another African Anacaridaceae member *Scelerocarya birrea* but lacking of sterols [13]. The major phytochemical content of tannins and flavonoids were reported from *Haematostaphis barteri* stem bark [14] and stem bark of *Nothopegia heyneana* [12]. Preliminary phytochemical screening of *Mangifera indica* resulted that the occurrence of phenols and flavonoids and lack of glycosides and sterols [15]. The total phenol content was high in the ethanol extract of stem bark (106.6 µg GAE/g) and it was followed by aqueous, chloroform and petroleum ether extracts (Table 3). There is a gradual increase in the phenol content with the increase of concentration of extract. The similar results were noted from the seed extracts of *Buchanania lanzan* [16] and also in *Nothopegia heyneana* [12]. Phenols are important constituent in herbal medicines which are possessing many kinds of pharmacological and biological properties like anti-oxidative, anti-allergic, antibiotic, hypoglycaemic and anti-carcinogenic [17]. The high content of phenolics in *N. travancorica* stem bark extracts indicates that this plant could be used for medicinal properties. The total flavonoid content of the four kinds of solvent extracts of stem bark at different concentration was measured that ethanol extract was showing good result of 63.32 µg Rutin/g (Table 4). Flavonoids are well known phytoconstituents in the family members of Anacardiaceae. The genus *Sponidas* has reported the presence of various flavonoids that is important secondary metabolites play a variety of biological role in plants including defence, UV protection, flower colouring and allelopathy [18]. The high content of flavonoids was observed from the leaves of *Anacardium occidentale* [19]. Phenolics and flavonoids are functioning as reducing agents, free radical scavengers and quenchers of singlet oxygen formation, apart from that they are playing important roles in the control of cancer and other human diseases [20]. Stem bark extract of *N. travancorica* for DPPH radical is scavenged by phytocompounds antioxidants through the donation of a proton forming the reduced DPPH. Various concentrations of the sample extracts (50 – 250 µ/ml) were mixed with 1.0 ml of solution containing DPPH radicals, resulting in the final concentration of DPPH being 0.2mM. The mixtures were the shaken vigorously and left to stand for 30 min, and the absorbance was measured at 517 nm. The percentage of inhibition in DPPH free radicals was observed with considerable activity when compared with standard Ascorbic acid (Fig. 2). The obtained DPPH radical scavenging property of the selected plant showed the positive attribution of its phytochemicals. The present results are compared with the antioxidant properties of *Lannea acida* of Anacardiaceae [21].

Table 1: Extraction characteristics of *Nothopegia travancorica* in different solvents

Types of Extracts	Plant part	Amount of Extract (gm)	Extract yield (%)	Colour of Extract
Petroleum ether	Leaves	7. 32	1.5	Dark green
	Stem bark	11.12	3.5	Dark brown
Chloroform	Leaves	8.22	2.1	Light green
	Stem bark	10.01	3.1	Dark green
Ethanol	Leaves	13.21	4.5	Dark green
	Stem bark	15.35	5.6	Black
Aqueous	Leaves	3.25	0.3	Pale green
	Stem bark	4.55	0.7	Dark brown

Table 2: Phytochemical screening of various extracts of *Nothopegia travancorica*

Chemical tests	Petroleum ether		Chlorofom		Ethanol		Aqueous	
	L	S.b.	L.	S.b.	L	S.b.	L.	S.b.
Alkaloids								
Mayer's reagent	-	-	-	-	-	-	-	-
Dragendroff' reagent	-	-	-	-	-	-	-	-
Wagener's reagent	-	-	-	-	-	-	-	-
Hager's reagent	-	-	-	-	-	-	-	-
Saponins								
Froth test	-	-	+	+	-	-	+	+
Sterols								
Salkowaski test	-	-	-	-	+	+	-	-
Libermann's reagent	-	-	+	+	+	+	-	-
Lieberman-Burchard's	-	-	+	+	+	+	-	-
Carbohydrates								
Mollisch's test	+	+	+	+	+	+	-	-
Fehling's test	-	-	-	-	+	+	-	-
Anthraquinones								
Borntrager's test	-	-	-	-	-	-	-	-
Cardioglycosides								
Lugal's test	-	-	-	-	-	-	-	-
Keller-Killians test	-	-	-	-	-	-	-	-
Tannins								
Lead acetate test	+	+	+	+	+	+	+	+
Ferric chloride test	+	+	-	+	+	+	+	+
Proteins								
Xanthoproteic test	-	-	-	-	-	-	-	-
Biuret test	-	-	+	+	+	+	-	-
Flavonoids								
Ammonia test	+	-	+	+	+	+	+	+
Alkaline reagent	+	+	+	+	-	+	+	+
Magnesium test	+	+	+	+	+	+	+	+

Table 3: Total phenol content in various extracts of *Nothopegia travancorica* stem bark

Concentration of Extract	% of phenol content µg (GAE/gDM)			
(µg/ml)	Petroleum ether	Chlorofom	Ethanol	Aqueous
100	13.6	12.1	23.4	10.2
200	21.2	22.2	40.3	30.6
300	25.3	34.3	68.8	63.4
400	40.2	51.7	80.1	70.5
500	42.8	65.2	106.6	72.3

Table 4: Total flavonoid content in various solvent extracts of *Nothopegia travancorica* stem bark

Concentration of Extract	% of phenol content µg (Rutin/µg)			
(µg/ml)	Petroleum ether	Chlorofom	Ethanol	Aqueous
100	1.0	8.18	11.25	0.0
200	0.13	15.30	22.32	0.0
300	2.15	17.27	30.13	0.62
400	4.83	27.52	38.52	10.25
500	8.11	36.38	63.32	21.03

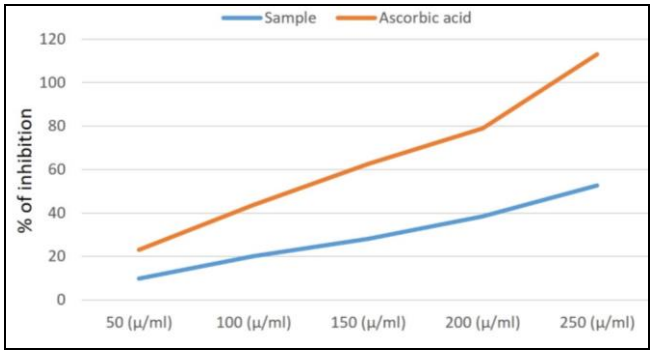

Fig 2: The percentage of the free radical DPPH by the antioxidant property of *N. travancorica* bark extract compared with standard Ascorbic acid.

Conclusion

The study is aimed to determine the phytochemical groups, total phenolic, flavonoid content and antioxidant property of an endemic plant *N. travancorica*. The obtained results could be useful for understanding knowledge on endemic plant and its phytochemicals and pharmacological importance. Further investigation on the sample species essentially needed for isolation and characterization of phytocompounds, if support the pharmacological evaluations and clinical trials of isolated phytocompounds towards the development of safer medicine to posterity.

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