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Preliminary Phytochemical Screening of an Endemic Plant *Nothopegia heyneana* Gamble (Anacardiaceae) in Southern India

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ABSTRACT: This study is aimed to investigate the phytochemical screening of different solvent extracts of leaves and stem bark of *Nothopegia heyneana* (Anacardiaceae). The results revealed that the presence of saponin, sterols, carbohydrates, tannins, proteins and flavonoids in the test extracts. Further quantification of total phenol and flavonoids from different solvent extracts of stem bark showed that high content of phenol (101.6 µg GAE/g) and flavonoid (67.34 µg Rutin/g) in ethanol extracts respectively. It confers the endemic plant possessed to have a rich source of phytocompounds for therapeutic properties which could be isolated and characterized for further development of safe medicine.

Key words: Endemic plant, *Nothopegia heyneana*, Phytochemicals, Phenolic and Flavonoids.

INTRODUCTION

Higher plants are sources of wide varieties of phytochemical constituents and which could be used as therapeutic agents and medicinal drugs that are used for treating many kinds of human illness since time immemorial. From the origin of human race, plants are continuously exploited for medicine and other purposes selectively by the human community based on their experience with plants in trial and error since long time. There has been available about 30,000 drug yielding plants worldwide and with more than 1,75,000 different known chemical compounds and phytomolecules screened for different purposes (Pullaiah, 2006). In India, about 8000 plants are 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. Until now many of the 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 the plant drug can be defined that is determined by identity, purity and content and other chemical, physical, biological properties or by the manufacturing processes. For quality identification 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 (Sivarajan and Balachandran, 2006). Drugs from the plants are easily available, less expensive, safe and efficient, which have less side effects. In contrast to synthetic single chemical therapeutics, phytomedicines are potentially beneficial through additive and synergetic action (Nasri and Shirzad, 2013).

Though plant drugs are carefully evaluated before medicinal use and are to be determined phytochemical entities and their properties. Usually the secondary metabolites such as alkaloids, flavonoids, phenolics, saponins, terpenoids and quinines are predominantly present in higher plants (Karuppusamy, 2009). They should be screened and characterized appropriately before therapeutic use. There are number of endemic plants until exists in wild, perhaps scientific knowledge not known their biological values. The present study is aimed to screen the phytochemical values of an endemic pant *Nothopegia heyneana* for its therapeutic importance.

MATERIALS AND METHODS

A. Collection of plant materials

The leaves and stem barks of *Nothopegia heyneana* Gamble (Fig. 1) were collected carefully from the semi-evergreen forest areas of Palni hills, on the way to Kodaikkanal, 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 and other environmental climatic changes until uses.

B. Extraction

Plant parts powders of 200 grams were extracted separately in Soxhlet's apparatus sequentially in 300 ml of Petroleum Ether, Chloroform, Ethanol and Aqueous. The process was run for 48 hrs after which the sample was concentrated using rotator evaporator and freeze dried to powdered form. 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.

Fig. 1. Flowering twig of *Nothopegia heyneana* Gamble.

C. Preliminary phytochemcial screening

The preliminary phytochemical tests were performed for testing different chemical groups present in extracts by standard phytochemical analysis (Harborn, 1998).

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_2SO_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 NH_3.

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 (Aiyegoro *et al*., 2010).

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. H_2SO_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.

D. Determination of total phenols

The total phenol content was determined by the Folin Ciocalteu procedure (Skerget *et al*. 2005) for ethanol extract of stem bark of *Nothopegia heyneana*. 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) (Soni *et al*., 2014).

E. Estimation of total flavonoid content

The total flavonoid content was determined by the aluminium chloride calorimetric 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 $AlCl_3.6H_2O$(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 (Chang *et al*., 2002).

RESULTS AND DISCUSSION

From leaves and stem bark of *N. heyneana* obtained good 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. heyneana* contained a significant amount of phytochemicals such as saponins, sterols, carbohydrates, tannins, proteins and flavonoids (Table 2). However, alkaloids, anthraquinones and cardiac glycosides could not be performed positively in qualitative tests. Tannins and flavonoids are strongly present in all four kinds of extracts from leaves and stem barks. The similar results were observed from the African Anacaridaceae member *Scelerocarya birrea* but lacking of sterols (Virginie *et al*., 2016). The major phytochemical content of tannins and flavonoids were reported from *Haematostaphis barteri* stem bark (Ezekiel *et al*., 2016).

Table 1: Extraction characteristics of *Nothopegia hyeneana.*

Types of Extracts	Plant part	Amount of Extract(gm)	Extract yield(%)	Colour of Extract
Petroleum ether	Leaves	8. 52	1.5	Dark green
	Stem bark	12.16	4.5	Dark brown
Chloroform	Leaves	9.42	2.1	Light green
	Stem bark	11.61	3.2	Dark green
Ethanol	Leaves	15.42	5.4	Dark green
	Stem bark	18.75	7.6	Black
Aqueous	Leaves	4.35	0.5	Pale green
	Stem bark	6.25	1.0	Dark brown

Table 2: Phytochemical screening of various extracts of *Nothopegia heyneana.*

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 heyneana* stem bark.

Concentration of Extract (µg/ml)	% of phenol content µg (GAE/gDM)			
	Petroleum ether	Chlorofom	Ethanol	Aqueous
100	15.6	15.5	25.2	22.8
200	22.5	26.2	42.6	40.5
300	30.4	38.3	71.5	66.4
400	41.8	52.7	88.2	76.5
500	51.5	61.8	101.6	92.7

Preliminary phytochemical screening of *Mangifera indica* resulted that the occurrence of phenols and flavonoids and lack of glycosides and sterols (Alshammaa, 2016). The total phenol content was high in the ethanol extract (101.6 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* (Khatoon *et al*., 2015). Phenols are important component in herbal medicines which are possessing many kind of pharmacological and biological properties like anti-oxidative, anti-allergic, antibiotic, hypoglycaemic and anti-carcinogenic (Stankovic, 2011). The high content of *N. heyneana* extracts indicates that this plant could be used for medicinal properties.

The total flavonoid content of the four extracts at different concentration was measured and was found that ethanol extract was showing good result that is 67.34 µg Rutin/g (Table 4). Flavonoids are known phytoconstituents in the family members of Anacardiaceae. The genus *Sponidas* has reported the occurrence of different flavonoids that is important secondary metabolites play a variety of biological role in plants including defence, UV protection, flower colouring and allelopathy (Pereira *et al*., 2015). The high content of flavonoids were observed from the leaves of *Anacardium occidentale* (Jaiswal *et al*., 2012). 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 cancet and other human diseases (Ghasemzadeh and Ghasemzadeh, 2011).

Table 4: Total flavonoid content in various extracts of *Nothopegia heyneana* stem bark.

Concentration of Extract (µg/ml)	% of phenol content µg (Rutin/µg)			
	Petroleum ether	Chlorofom	Ethanol	Aqueous
100	0.0	10.28	18.25	0.0
200	0.15	16.31	25.36	0.0
300	2.22	32.42	31.12	2.64
400	6.82	40.56	43.51	11.25
500	10.12	52.41	67.34	24.02

CONCLUSION

The basic aim of the research was to determine the phytochemical spectrum, total phenolic and flavonoid content of an endemic plant *N. heyneana*. It could be useful for understanding knowledge on endemic plant and its phytochemical importance. Further investigation on isolation and characterization of phytocompounds, if support the pharmacological and clinical evaluation of isolated compounds towards the development of safer medicine for posterity.

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