ISSN:0975-1459
P.Devi et al /J. Pharm. Sci. & Res. Vol.1(3), 2009, 117-122.
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Phyto-Physico chemical evaluation and Antioxidant activities of leaves of *Naphellium lappaceum*

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ABSTRACT
The present study deals with the preliminary phyto physicochemical evaluation of leaf *Naphellium lappaceum.* The standardization is carried out on the basis of physiochemical and phytochemical studies including parameters such as fluorescence characteristics and analysis of inorganic constituents. The study contributes to the development of standardization parameters of herbal drugs used in Indian system of medicine. Thin layer chromatography has been carried out for all the phytochemical constituent and the inference is noted. It was found that Tannins, Phytosterols, Flavanoids, Saponins, Triterpenoids and Cardiacglycosides present in the extracts.The antioxidant activity was determined by two invitro methods – DPPH and H_2O_2 radical scavenging .
Key words-Antioxidant activity, *Naphellium lappaceum*, Phyto-Physiochemical evaluation

INTRODUCTION

Naphellium lappaceum is a medium sized tree, grown in Kallar on the lower slopes of Nilgiris. The fruits are considered stomachic , anthelmintic , used as astringent and febrifuge . seeds are bitter and narcotic,leaves are used in poultices for headache.

Macroscopy

Leaves are pinnate, leaflets elliptic, obovate, flowers polygamous, small, white in panicles. Fruits globous, densely covered with soft, fleshy, yellow to bright red spines, pericarp thin, leathery. Seeds are oblong, arillate. Aril white, translucent, juicy and acidulous.

MATERIAL AND METHODS

Extraction

The leaves of the plant was washed under running tap water to remove adhered dirt, followed by rinsing with distilled water, shade dried and pulverized in a mechanical grinder to obtain coarse powder. The dried powdered leaf material (250g) defatted with petroleum ether and extracted with 3 times with 70% acetone ,ethyl acetate, n-butanol, at room temperature. The extract was concentrate under reduced pressure at below 40.8°C.The aqueous extract of the leaf material was refluxed with distilled water for 3 hours. Following filtration and concentration under vacuum the residue was obtained (28g) which was preserved in a desiccator for further use.

Physico- Chemical standards

Physico- chemical parameters of the powdered drug such as ash value, extractive value, loss on drying and crude fiber content were performed according to the method [3,4,5] Extracts were prepared by various solvents by standard methods and percentage of dry extract was calculated in terms of air-dried leaf powder. (Table 1, 2, 3)

Fluorescence characteristics

When physical and chemical parameters are inadequate as it often happens with the powdered drugs, the plant material may be identified from their adulterants on basis of fluorescence study [6,7] (Table 4)

Behaviour of le af powder with different chemical reagents

Behaviour of leaf of *Naphellium lappaceum* with different chemical reagents was performed to detect the occurrence of phytoconstituents along

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Table 1: Ash values

S. No.	Type of ash	Results
1.	Total ash	9 % w/w
2.	Acid insoluble ash	3.1 % w/w
3.	Water soluble ash	2.7 % w/w

Table 2: Extractive value, Percentage yield and colour of extracts

Solvent used	Percentage yield	Colour of extract
Petroleum ether	12	Green
Acetone	6	Brownish green
Ethyl acetate	7.2	Green
Butanol	6.5	Dark brown
Water	8.6	Brownish black

Table 3. Loss on drying

Loss on drying	4.2% w/w

Table 4: Fluorescence characteristics of leaf extract of Naphellium *lappaceum*

Powder + Reagent	Color observed in Ordinary light	Color observed under Ultra violet light Short (254 nm)	Color observed under Ultra violet light Long (365 nm)
Powder	Brown	Green	Green
Powder+ 1N NaOH in methanol	Brownish black	Green	Green
Powder+ 1 N NaOH in water	green	Green	Black
Powder++ 1 N HCl	Brownish yellow	Green	Black
Powder+50% HNO_3	Dark Green	Light Green	Black
Powder+50% H_2SO_4	Brown	Green	Black
Powder+Methanolic NaOH.dried+ nitrocellulose in acetic acid	Yellowish green	Dark Green	Black
Powder+ 1N NaOH + nitrocellulose in aceticacid	Dark brown	Light Green	Greenish Black

with colour changes under ordinary daylight by standard method [8] (Table 5)

Quantitative standards

Total carbohydrate content in leafs of *Naphellium lappaceum* by phenol-sulphuric acid method [9] was estimated to be 13.25%w/w. Similarly protein content[10], was found to be, 0.857%w/w respectively.
(Table 6)

Determination of Saponin

According to the results obtained from positive foaming test and high foaming index [11] of leafs of *Naphellium lappaceum* study was carried out for the estimation of total saponin content [12,13]. (Table 6).

Preliminary ph ytochemical investigation

The qualitative chemical test of various extracts of *Naphellium lappaceum* was carried out using standard procedure [14,15,16,17]. Carbohydrates, proteins and amino acids are present in butanol and aqueous extract. Saponin, flavanoids and glycosides are present in ethyl acetate and butanol extract. Phyto sterols, and phenolic compounds are present in ethyl acetate, butanol and aqueous extract.

Thin Layer Chromatography

About 30gms of silica gel – B was weighed out and it was shaken with 100ml of water to form a homogenous suspension. The suspension was poured into a thin layer chromatography applicator which was adjusted to 0.25mm thickness. 20 to 40 Carrier plates (20.5cm) were laid down for air drying. The plates were kept in the hot air oven at 110°C for one hour to activate the silica gel – G. The plates were stirred in a dry atmosphere and used whenever required. By using the capillary tube the extracts are spotted on the T.L.C plates 2cm above the bottom and in the chromatogram in various solvent systems for different compounds. The spots are developed in solvent system were identified by means of different spraying reagents. (Table 7).

HPTLC fingerprinting of d ifferent extracts of *Naphellium lappaceum* [18]

Butanolic extract

10µl of 1mg/mL solution of butanolic extract in methanol was applied on the silica gel GF254 HPTLC plates (10x10). Chloroform: Acetone: Formic Acid (9:2:1) was used as the mobile phase. After development the plates were scanned in ultraviolet range at 254 nm and 366nm and then the plates were derivatized by using 20% ethanolic sulphuric acid. After spraying four spots were observed at R_f 0.22, 0.35, 0.39, 0.44.

Petroleum ether extract

10µl of 1mg/mL solution of Petroleum ether extract in acetone was applied on the silica gel GF254 HPTLC plates (10x10). Petroleum ether: ethyl acetate: acetone (9:0:5::0:5) was used as the mobile phase. Six spots at Rf 0.32,0.36, 0.42, 0.58,0.66,0.69 were observed under visible after spraying the plates with 20% ethanolic acid reagent.

Evaluation of in v itro a ntioxidant activity

Diphenylpicrylhydrazyl (DPPH) radical scavenging activity, Hydrogen peroxide (H_2O_2) radical scavenging activity was determined by following procedures [19,20,21,22]

DPPH radical scavenging activity: 0.1mm solution of DPPH in ethanol was prepared and 1 ml of this solution was added to 0.3 ml of extract solution in water at different concentrations (10-100µg/ml) .Thirty minutes later, the absorbance was measured at 517 nm. Lower absorbance of the reaction

Table 5: Behaviour of leaf extract of *Naphellium lappaceum*

Reagent	Colour / ppt	Constitituent
Powder	Green	
Powder + con. H_2SO_4	Brown	Carbohydrate present
Powder + aqueous $FeCl_3$	Bluish black	Tannin present
Powder + Iodine solution	No black	Starch absent
Powder + Aqs. HgCl2	blue	Alkaloids present
Powder + picric acid	Colour change	Alkaloids present
Powder + Mg HCl	Black colour	Flavonoids present
Powder + aqueous $AgNo_3$	Precipitate is formed	Protein present
Powder + ammonia solution	Pink colour	Cardiac glycoside present
Powder + Aqs. KOH	Pink colour	Cardiac glycoside present
Powder + Aqs. Na nitride	Red colour	Phytosterols present
Powder + Water (shaking)	Foam is produced	Saponins present

Table 6: Results of Quantitative estimation of leaf extracts of *Naphellium lappaceum*

S.No	Estimation		Results
1.	Foaming index		More than 1000
2.	Total saponin content	Method I	8.5% w/w
		Method II	9.5 % w/w
3.	Total carbohydrates		10.5 % w/w
4.	Total proteins		0.557 % w/w
5.	Total crude fibers		2.6% w/w

Table 7: Phytochemical constituents of Thin Layer Chromotography

S.No	Active constituents	Mobile phase	Spraying reagent	Inference
1.	Cardiac Glycosides	Ethyl acetate :Hexane 3:7	Anisaldehyde Sulphuric acid	Orange
2.	Triterpenes	Petroleum ether : Dichloroethane : Acetic acid 12.5:12.5:2	Chloro sulphonic acid reagent	Dark brown
3.	Phytosteroles	Hexane : Diethyl ether 32:1	Stannic chloride reagent	Orange brown round
4.	Carbohydrates	Butanol : Aceticacid : Water : Ether 9:6:1:3	Phenol sulphuric acid	Greenish brown

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mixture indicates higher free radical scavenging activity. The capability to scavenge the DPPH radical and the standard (BHT) was calculated using the following equation.

$$\text{DPPH Scavenged}\,(\%) = \frac{A_{cont} - A_{test}}{A_{cont}} \times 100$$

Where A_{cont} is the absorbance of the control reaction and A_{test} is the absorbance in the presence of the sample of the extracts.

***H_2O_2 radical scavenging activity*:**

A solution of H_2O_2 was prepared in phosphate buffer (pH 7.4). H_2O_2 concentration was determined spectroscopically measuring absorption with extinct coefficient for H_2O_2. Different concentrations of the extracts in distilled water were added to a H_2O_2 solution (0.6 ml, 40 mM). Absorbance of H_2O_2 at 230 nm was determined 10 min later against a blank solution containing the phosphate buffer without H_2O_2. Ascorbic acid was used as the standard. The % of H_2O_2 scavenging was calculated by the equation:

$$H_2O_2\ \text{Scavenged}\,(\%) = \frac{A_{cont} - A_{test}}{A_{cont}} \times 100$$

Where A_{cont} is the absorbance of the control reaction and A_{test} is the absorbance in the presence of the sample of the extracts.

RESULTS AND DISSCUSSION

The preliminary phytochemical screening of the extract showed the presence of tannins, saponins and flavanoids in the extracts of *Nephellium longan.*The extract exhibited scavenging potential with IC_{50} value of 14.22 µg/ml and 20.25 µg/ml for DPPH and H_2O_2 radicals respectively. (Table 8,9).The extract showed dose dependant increase in reducing power that was comparable to standard BHT. In the present days of modernization, Ayurveda no longer can afford to remain confined to use of conventional norms of medication. It has to accept the new challenges and be prepared to answer the queries of the modern man about the quality and efficacy of the herbal drugs administered to him and also how they are collected , processed, preserved and used. The above studies provide information in respect of their identification, chemical constituents and physico chemical characters which may be useful standardization of herbal drugs of folk medicinal practice of present era and enrichment of Ayurvedic Pharmacopoeia.

Table 8. DPPH radical scavenging activity

Drug Concentration	DPPH Scavenged %	
	BHT	*N. longan*
10 µg/ml	23.4±0.11	13.9±0.17***
20 µg/ml	52.9±.17	54.8±0.78***
50 µg/ml	63±1.4	66.8±1.1***
100 µg/ml	70.33±1.24	78.45±1.14**
IC_{50}	14.22µg/ml	20.25µg/ml

Values are mean ± S.E., n = 5; data analysed by one way ANOVA And student's "t" test; **p< 0.001, *** p<0.0001 Vs control.

Table 9. H_2O_2 radical scavenging activity

Drug Concentration	H_2O_2 Scavenged %	
	Ascorbic acid	*N.longan*
50 µg/ml	23.17±0.25	12 ±0.19***
100 µg/ml	44.24±0.62	24.58±.75**
200 µg/ml	66 .26±1.01	38.26±1.11***
300 µg/ml	66.62±1.54	48.89±1.18**
350 µg/ml	73.3±2.13	55.43±1.40***
IC_{50}	93.6µg/ml	99.73µg/ml

values are mean ± S.E., n = 6; data analysed by one way ANOVA and student's "t" test; **p< 0.001, *** p<0.0001 Vs control.

CONCLUSION

The present study on physicochemical characteristics and preliminary phytochemical screening of provide useful information which may help in authenticating the genuine plant along

with nature of phytoconstituents present in it.

REFERENCES

[1] *The Wealth of India*, Raw Materials, Publication and Information Directorate (CSIR), New Delhi 2003,14-15.

[2] Chopra, R.N. *Glossary of Indian Medicinal Plants*,CSIR,1956,113

[3] Anonyums. *The Ayurvedic Pharmacopoeia of India*, Ministry of Health and Family Welfare , NewDelhi, 1996, A-53-A55.

[4] Anonyums. *Pharmacopoeia of India*, Ministry of Health and Family Welfare,The Controller of Publication , NewDelhi, 1996, A-47-A89.

[5] Khandalwal, K.R. *Practical Pharmacognosy Techniques and Experiments*. Nirali Prakashan, 1999, 146-148.

[6] Pratt RJ , Chase CR .J.Am.Pharm.Ass .1949; 38: 324-333.

[7] Kokoski J, Kokoski R and Salma F.J. J.Am.Pharm.Ass . 1958; 47: 715.

[8] Singh, V.K, Govil Gurdip Singh , *Recent progress in Medicinal plants* ,SCI Tech publishing LIC Texas, 325.

[9] Dubois M, Gilles KA, Hamilton JK , Rebers PA and Smith F ,Colorimetric method for determination of sugars and related substances. Analytical chemistry. 1956; 28: 350-356.

[10] Haritee EP ,Determination of protein ,A modification of Lowrys method , that gives a linear photometric response , Analtyical Biochemistry. 1972; 48: 422-427.

[11] WHO, *Quality Control for Medicinal Plant Material*, AITBS Publishers, New Delhi Geneva 1998,46.

[12] Brueton, J, *Pharmacognosy, Phytochemistry, Medicinal Plants* ,Intercept Ltd., Paris 1999, 310-386.

[13] Rajpal, V, *Standardization of Botanicals*, Eastern Publishers, NewDelhi 2002 41.

[14] Trease, G.E and Evans, W.C ,*Textbook of Pharmacognosy*, 12th ed. Belleire, Tindal, London 1983; 343-383.

[15] Kokate, C. K, *Practical pharmacognosy* , 4th ed, Vallabh Prakashan Pune 1996,107.

[16] Harbone, J.B. *Phytochemical methods*,Chapman and Hall, London ,1973.

[17] Plaisted ,Philip ,H, Contributions from Boyee Thompron Institute . 1958; 9: 231-44.

[18] Sethi, P.D, *HPTLC Quantitative Analysis of Pharmaceutical Formulations*, 1st ed. CBS Publishers and Distributors, NewDelhi 1996, 3-73.

[19] I.C.Lu, Y.W.Chen and C.C.Chou ,*Journal of Food Drug Analysis*. 2003, 2, 277-282.

[20] N.Benkeblia ,*Brazilian Archievs of Biology and Technology*. 2005,48, 753-759.

[21] Ilhami GULCIN , H.A.Alici and M.Cesur,*Chem.Pharm.Bull*.2005, 3, 281-285.

[22] Pilar Prieto, Manuel Pineda and Miguel aguliar.*Analytical Biochem*.1999,269,337-341.