RESEARCH ARTICLE

GC- MS ANALYSIS OF METHANOLIC EXTRACT OF NEEL (*INDIGOFERA TINCTORIA*) ROOT

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Abstract: *Indigofera tinctoria* L. is famous as Indian indigo plant from early periods. It belongs to the family Leguminosae and is one of the oldest colouring agents known to man. Neel leaves are used in ayurveda and it forms a major ingredient of preparations like Neelibringadi oil, Aravindasavam, Neelithulsyadi kashayam etc. The Juice of the Neel leaves mixed with honey is effectively used against enlargement of liver and spleen, epilepsy and other nervous problems. The root of the plant which was usually abandoned after harvest also has very good therapeutic potentials reported. It is being used by traditional healers and also in folk medicines. Decoction of the root is given to treat calculus. An infusion of root is given as an antidote in cases of arsenic poisoning. Root infusion is used as an antidote against snakebites and to treat insect and scorpion stings. It is an antidote against mushroom and arsenic poisoning Root preparation is used in Tanzania as a remedy against syphilis, gonorrhea and kidney stone. In the present study, phytochemical investigations were carried out in the roots of Neel. The methanol extract of root when subjected to GC-MS analysis revealed the presence of many compounds which are reported to have pharmacological activities supporting the medicinal usages of this root. The specific TLC fingerprint developed can also be made useful for authentication of Neel root when used as raw herbal drug.

Key words: *Indigofera tinctoria*, Neel, GC-MS analysis

INTRODUCTION

Indigofera tinctoria from the family Leguminosae, is famous from ancient time as a source of blue dye called as Indian indigo. It is one of the oldest colouring agents known to man. Dye is obtained from the processing of the plant's leaves .This plant is known as Neelamari in Malayalam, Neel in Hindi and Neelini in Sanskrit. In India, it is found almost throughout and cultivated in the states of Assam, Andhra Pradesh, West Bengal, Bihar, Tamil Nadu and Kerala. It is a branching shrub growing up to 2 m height. Neel is not only a dye source but also used for medicinal purposes. In Ayurvedic system of medicine, *Indigofera tinctoria* leaves are used as a major ingredient of Neelibhringadi oil used for the promotion of hair growth and in Neeli thulasiadi thailam, Neeli thulasiadi kashayam ,Aravindasavam etc. The Juice of leaves mixed with honey is effectively used against enlargement of liver and spleen, for epilepsy and nervous problems (Kirtikar *et al*., 2008 ;Nambiar *et al*., 2000 and Warrier *et al*., 1997).

The root of the plant which was usually abandoned after harvest also has very good therapeutic effects reported. Neel root is especially used in traditional and folk medicine. Root infusion is used as an antidote against snakebites and to treat insect and scorpion stings. It is an antidote against mushroom and arsenic poisoning. Tribals use root decoction internally for kidney stone, use root /leaf juice internally for snake poison, rat poison, etc. In Cameroon, the twigs and the roots have been widely applied as a treatment for toothache. Root preparation is used in Tanzania as a remedy against syphilis, gonorrhea and kidney stone. (Arun *et al*.,2007;Tomar,2007;Tomar,2009;Venkitachalam, 2018 and Sugandhi *et al*.,.2019).

Scientific evidences are scarce linking of the indigenous knowledge to the therapeutic potentials. Information if drawn will help for better utilization of many valuable plant resources especially used in folk medicines.

In the present study, phytochemical investigations were carried out in the roots of Neel. The methanol extract of root was subjected to GC-MS analysis for detection of various volatile compounds present. Many compounds present are reported to have pharmacological actions supporting the medicinal usages of this plant root. The specific TLC fingerprint developed will be help in the correct identification of the genuine root samples.

MATERIALS AND METHODS

Indigofera tinctoria (Neel) plant samples were collected from Viswanathan Memmorial Herbal Garden of Kerala Agricultural University, Vellanikkara and authenticated by botanist. The root portion was cut apart, washed, cleaned, dried and powdered for doing analysis. 10% methanol extract as well as aqueous extract (hot extraction method) of the root were prepared using standard procedure. Aqueous extract was subjected to qualitative chemical investigation for identification and estimation of different phyto constituents using

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standard procedures (Debiyi. *et al* ., 1978; Hey *et al* ., 1987 and Horborne,1998) and the methanol extract was also used for GC-MS analysis and for developing TLC fingerprints.

For developing TLC fingerprints various mobile system combinations and spray reagents were tried (Wagner *et al*.,1996) and the best one was selected. Silica gel $_{60}$ F $_{254}$ sheets were used as solid phase. The chromatogram developed using Toluene: Ethyl acetate and acetic acid in the ratio 7.5:2.5:0.06, as mobile phase was found to be good with specific bands under UV 365nm**.**

GC-MS analysis of plant extract reveals the presence of important volatiles present in the plant (Pavitra *et al* .,2012)In the present study GC-MS analysis of methanol extract of the Neel root was carried out on a GC-MS equipment Model 7890A GC with 5975C with triple axis detector. Experimental conditions of GC-MS system were as follows: Column: DB 5 MS 30m X 0.250 mm Diameter X 0.25 micrometer thickness. Flow rate of mobile phase (carrier gas: He) was set at 1.0 ml / min. The temperature programme (oven temperature) was 40°C raised to 280°C at 5°C/min rate and injection volume was 3 µl, pressure 7.0699 psi, injection mode split. Results were compared by using NIST 08 spectral data library. Using Dr.Duke's phytochemical and ethanobotanical database (online), the biological activity of the identified phyto compounds were ascertained.

RESULTS AND DISCUSSION

The results of phytochemical screening of the extract of Neel root is presented in Table 1. The plant extract when subjected to standard chemical tests for detection as well as estimation of various compounds revealed that it contains all major biochemicals like phenols (0.1%), tannins (0.05%), flavonoids (55mgRE/g), carbohydrate(3%), starch(0.43%), protein(0.16%), except saponin and steroids which were found absent. Root contained Indican (0.3%) also which is a precursor to indigotin. The antioxidant activity was observed as 2461 ug AAE/g. (Table.2.)

TLC chromatogram (Figure 1) developed using toluene : ethyl acetate and acetic acid in the ratio 7.5:2.5:0.06 as mobile phase in solid phase Silica gel 60 F $_{254}$ when viewed under UV-365 nm revealed 6 specific flourescent bands with different Rf values as 0.93,0.48,0.35,0.32 (Flourescent blue coloured) and 0.73 and 0.42 (Flourescent green coloured).

The plant root methanol extract when subjected to GC MS analaysis revealed the presence of 13 different phyto compounds (Table.3 and Figure 2). Prevailing compounds were squalene (15.12 %), Methyl 2-hydroxy-eicosanate (10.12%), 2-Propanol, 1-bromo- 3-Ethyl-2-pentanol (13.24%) and racepinephrine (7.24%). Many of the constituents detected were reported to have biological activities like antioxidant, antimicrobial, anti inflammatory, antifungal, anti cancer, hypocholesterolemic and muscle relaxant properties as detailed in the Table (3) which revealed the drug potential of Neel root and justified various folk medicinal uses of this root.

Table 1. Phytochemical screening of Neel root extract

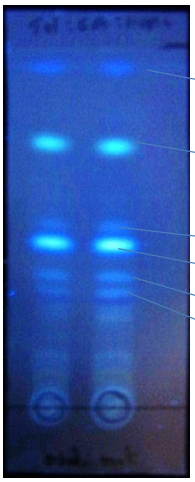
	Flavanoid	**Steroid**	**Glycoside**	**Alkaloid**	**Phenol**	**Saponin**	**Triterpenoid**	**Tannin**
Neel root	+	-	+	+	+	-	+	+

+ Present, - absent

Table 2. Phytochemicals estimated in Neel root extract

Compound /activity	**Content**
Phenol content (%)	0.10
Tannin (%)	0.048
Total starch (%)	0.43
Total carbohydrate (%)	3.00
Total protein (%)	0.16
Indican content(%)	0.30
Flavonoids (mgRE/g)	55
Anti oxidant activity (ug AA /g)	2461

TLC fingerprint of Neeli root – Figure 1



Rf	Colour
0.93	Flourescent blue
0.73	Flourescent green
0.48	Flourescent blue
0.42	Flourescent green
0.35	Flourescent blue
0.32	Flourescent blue

Neel root
Methanol extract

Tol:EA;HOAC 7.5:2.5: 0.06 ,Silica gel 60 F_{254}

Table 3. Phytocompounds detected in the methanol extract of *Indigofera tinctoria* (Neel) root by GC-MS

Sl. NO:	Compound detected	Retention time (RT)	Area %	*Reported biological activity of compound
1	Ethylamine (2-diethylboryloxy-Actinobolin Benzeneethanamine, 2-fluoro-.beta.,3-dihydroxy-N-methyl-)	1.338	2.74	
2	Methyl 2-hydroxy-eicosanate (Z-8-Methyl-9-tetradecen-1-ol formate Methyl 7,10-hexadecadienoate)	1.495	10.12	
3	2-Propanol, 1-bromo- 3-Ethyl-2-pentanol Propanoic acid (2-hydroxy-, methyl ester)	1.624	13.24	
4	Hexadecanoic acid(methyl ester)	37.232	3.25	Antioxidant, antiinflammatory ,hypocholesterolemic , nematicide, pesticide, antiandrogenic flavor, hemolytic,5-Alpha reductase Inhibitor , potent mosquito larvicid
5	8-Azabicyclo[4.3.1]decan-10-one (8 -methyl- Benzenemethanol, 3-hydroxy-.alpha. -[(methylamino)methyl]-, (R)-Methylpent-4-enylamine Phenol, 4-(2-aminopropyl))	40.421	3.48	
6	Racepinephrine	40.555	7.24	Muscle relaxant, bronchodialator
7	1,2-Benzenediol (4-(2-amino-1-hydr oxypropyl)-Benzenemethanol (3-hydroxy-.alpha.-[(methylamino)methyl]-, (R)-)	46.242	1.95	
8	2-Methylaminomethyl-1,3-dioxolane Benzyl alcohol, (alpha.-(1-aminoet hyl)-m-hydroxy-, (-)- Urea, butyl)	47.787	1.26	
9	Benzenemethanol (3-hydroxy-.alpha. -[(methylamino)methyl]-, (R)- Azetidin-2-one	47.848	2.46	Antioxidant

	3,3-dimethyl-4-(1-aminoethyl)			
10	Cyclotrisiloxane (hexamethyl- Silane, 1,4-phenylenebis[trimethyl Benzo[h]quinoline, 2,4-dimethyl-)	51.428	0.98	Antimicrobial
11	Silane (1,4-phenylenebis[trimethyl 2-Ethylacridine)	51.659	0.80	
12	6-Octadecenoic acid, (Z)- Octadec-9-enoic acid 11-Octadecenoic acid, methyl ester	51.771	0.43	Anti-cancer
13	Squalene 2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, Tetracosahexaene	51.921	15.12	Emollient , antioxidant, antitumor activities.
*Source: Dr.Duke's phytochemical and ethanobotanical database				

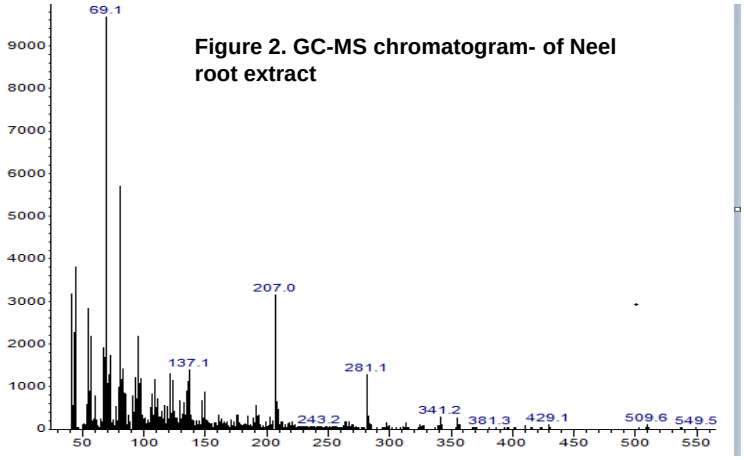


Figure 2. GC-MS chromatogram- of Neel root extract

CONCLUSION

From these results, it could be concluded that *Indigofera tinctoria* root contains various bio-active compounds which justifies the use of this root to treat various ailments by traditional and folk medicine practitioners. The specific TLC fingerprint developed will be helpful for doing the correct identification of the genuine root samples as a herbal raw drug.

However, isolation of individual phytochemical constituents and testing it clinically for specific biological activity will be definitely giving fruitful results and will open up a new area of investigation of individual components and their pharmacological potency for which further research is required.

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