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Phytochemical Screening and Medicinal Potential of *Phyllanthus niruri*

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Abstract

Medicinal plants have been discovered and used in traditional medicine practices since prehistoric times. Plants synthesize hundreds of chemical compounds for functions including defense against insects, fungi, diseases and herbivorous mammals. *Phyllanthus niruri* is a small herb distributed through the tropical and subtropical regions of both hemispheres. It is predominantly known for its anti-jaundice property. In this study, an attempt was made to reveal its medicinal property by studying its phyto-constituents by qualitative, quantitative and GC-HRMS analysis. This study discovered *P. niruri* deposits very high percent concentration and diversity of antimicrobial compounds which may govern its property to fight against jaundice and may also have potential to tackle other microbe borne diseases.

Keywords - *P. niruri*, antimicrobial, Hepatoprotective, Octaecanoic acid.

1. Introduction

Humans live in association with diverse populations of bacteria, fungi, viruses and Archaea [1]. The microbial world is vast, diverse, and dynamic and they represents the large component of the planet's biomass. Microorganisms have colonized virtually every environment on earth ranging from deep sea thermal vents, polar sea ice, desert rocks, guts of termites, roots of plants, to the human body. Much as we might like to ignore them, microbes are present everywhere in our body, living in our mouth, skin, lungs, and gut. Indeed, the human body has 10 times as many as many microbial cells as human cells. They are the vital part of our health, breaking down otherwise indigestible foods, making essential vitamins, and even shaping our immune system [2]. Many microbes live in the symbiotic association with the human but it is not yet clear what range of normal interactions should be to convert this interaction into beneficial, harmful or neutral. Thus microbe largely influence the host health or disease [3].

Microbes cause harmful effects on human health as well. The human society is always facing the problems of infectious diseases. The worldwide efforts for prevention and cure of these deadly infections turning out to be very difficult and in many cases it is not even possible to handle the

infections and thus they remain major causes of human morbidity and mortality.

Medicinal plants harbors huge diversity of active secondary metabolites like alkaloids, phenols, tannins, glycosides, sterols, terpenes, etc. which represent a rich source of antimicrobial compounds. Use of plants in different countries have resulted in the development of many potent and powerful drugs [4]. The raw extract of these medicinal plant parts is used as a drug and it shown to possess varied medicinal properties. The different parts used include root, stem, flower, fruit, twigs exudates and modified plant organs. On the other hand and most importantly, these raw drugs are collected in smaller quantities by local communities and folk healers for local use, many other raw drugs are also collected in larger quantities and traded in the market as the raw material for many herbal industries [5].

Plants contribute significant source of therapeutics or curative aids and thus have key role in maintaining the world health. Plants produces hundreds of chemicals known as the secondary metabolites which functions in their defense by fighting against bacteria, fungi, insects and herbivore animals. These secondary metabolites also plays a very crucial role in human health. Numerous plant products containing useful secondary metabolites are commercialized for the betterment of the patients belonging to nearly all spectrum of diseases. According to the WHO reports, as many as 80% of the world's population depends on the traditional medicine for their primary healthcare needs [6].

P. niruri is mainly known for its hepatoprotective activity. The use of this plant dates back to over 2000 years in the Ayurvedic medicines. In Ayurveda it is used in ailments like jaundice, , frequent menstruation, gonorrhea and diabetes and in poultice for skin ulcers, sores, swelling, and itch [7]. HIV-1 cells that cultured on MT-4 cell lines when treated with alkaloidal extract of *Phyllanthus niruri* showed promising activity [8]. More over this plant is also known to have hypotensive, antioxidative, hypoglycemic, diuretic and anti-inflammatory activity [9]. This study was carried out to quantify the secondary metabolites and to identify the bioactive compounds that are responsible for such activities.

2. Material and Method

The plant of *P. niruri* was collected from Rashtrasant Tukadoji Maharaj Nagpur University Campus locality, North Ambazari road Nagpur, Maharashtra, India.

For the plant part used for the analysis is leaves which are harvested from the plants and washed thoroughly by distilled water before use, shade dried, powdered by grinder and powder was used for the analysis.

2gm of powder was macerated in 10ml each of methanol, ethanol and petroleum ether at room temperature for 24hrs, filtered with Whatman no. 2 filter paper and used for the phytochemical analysis.

The phytochemical analysis in present investigation carried out in four steps. In the first step, phytochemical screening was performed to qualitatively analyze the presence or absence of the major groups of secondary metabolites form the *P. niruri*. In the next step, the quantitation was done to estimate the abundance of the particular group of secondary metabolite. In the third step, the GC-HRMS analysis of the plant was done to know the compounds present in the ethanolic extract of the plant. And lastly in the fourth step, the identified compounds by the GC-HRMS studies was literature reviewed for knowing the exact medicinal potential of each compounds.

1. Phytochemical screening

The alkaloid, phenol, tannin, flavonoids, terpenoids, glycosides, steroids and saponins were done by the methods given in standard literature (Harborne, 1973, 1984 and 1998). Multiple test was carried out for the additional confirmation of the test. Methanol, ethanol and petroleum were

used for solvent system.

2. Quantitative Test

Quantitation was done for alkaloid, phenol, tannin and flavonoid according to the methods given in standard literature (Sadashivan and Manikam, 1996).

3. GC- HRMS

Gas chromatography with high resolution Mass Spectrometry (make of MS-Jeoul, model-AccuTOF GCV) was used for identifying the compounds present in the ethanolic leaf extract of *P. niruri*.

3. Result and Discussion

Preliminary phytochemical screening shown the presence of alkaloid, phenol, tannin, terpenoids, steroid, saponins ethanolic extract whereas flavonoid shown its absence. The methanolic extract of *P. niruri* shown negative test for saponins and steroids whereas positive test was shown for the alkaloid, phenol, tannin, flavonoid and terpenes. Liebermann's test for glycosides gave the positive results but the negative result shown in Bomtrager's test. Phenol, tannin, glycosides, steroids, and saponins is absent in Petroleum extract. Flavonoid and terpenoids is present in the petroleum extract. Mayer's test for the alkaloid shown positive results whereas Dragendorff test gave the negative result for the alkaloids (Table 1).

Table No. 1: Preliminary Screening of *Phyllanthus niruri*

Sr. No	Chemical Test	Ethanol Extract	Methanol Extract	Petroleum ether Extract
1	**Alkaloid**			
	Mayer's test	+	+	+
	Dragendorff test	+	+	-
2	**Phenol**			
	Ferric Chloride test	+	+	-
3	**Tannin**			
	Ferric Chloride test	+	+	-
4	**Flavonoid**			
	Shinode test	-	+	+
5	**Terpenoids**	+	+	+
6	**Glycosides**			
	Liebermann's test	+	+	-
	Bomtrager's test	+	-	-
7	**Steroids**			
	Salkowski test	+	-	-
8	**Saponins**			
	foam test	+	-	-

The quantitation of four major secondary metabolites were done. This includes alkaloid, phenol, tannin and flavonoid. The maximum amount of tannin (5.74mg/gm) was observed in the leaf extract. Phenol content was found in minimum concentration (0.034mg/gm) as compared to rest of the tree secondary metabolites. Significant concentration of flavonoids (1.134mg/gm) was also found to be present in the extract.

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Table No. 2 : Quantification of Secondary Metabolites

Plants Species	Alkaloid	Phenol	Tannin	Flavonoid
P. niruri	0.250mg/gm	0.034mg/gm	5.74mg/gm	1.134mg/gm

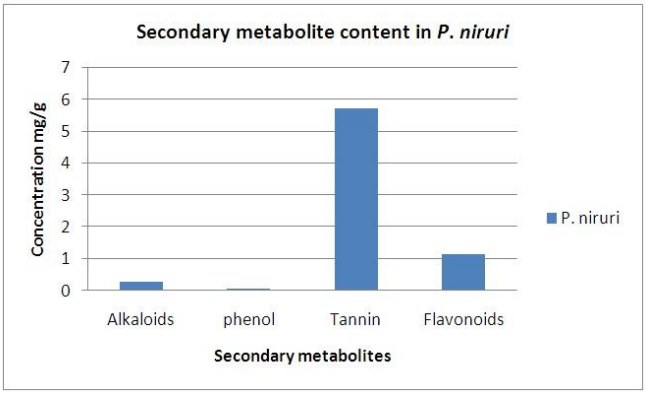

Figure No. 1

Gas Chromatography- High resolution mass spectrometry gave the signals for the presence of twelve compounds. Maximum of which is of fatty acid in nature. Percent peak area is the measure of the comparative concentration of particular compound. Maximum percent peak areas was found to be of octadecanoic acids, methyl ester (57.24%) and of 1, 2, 3-Denzenetriol (43.71%). Significant percent peak area of 9, 12, 15 – octadecatrienoic acid (Z,Z,Z), 19,12,15-octadecatrienoic acid, methyl ester (Z,Z,Z) and n – Hexa decanoic acid was observed i.e. 17.71%, 21.18 and 25.59 respectively.

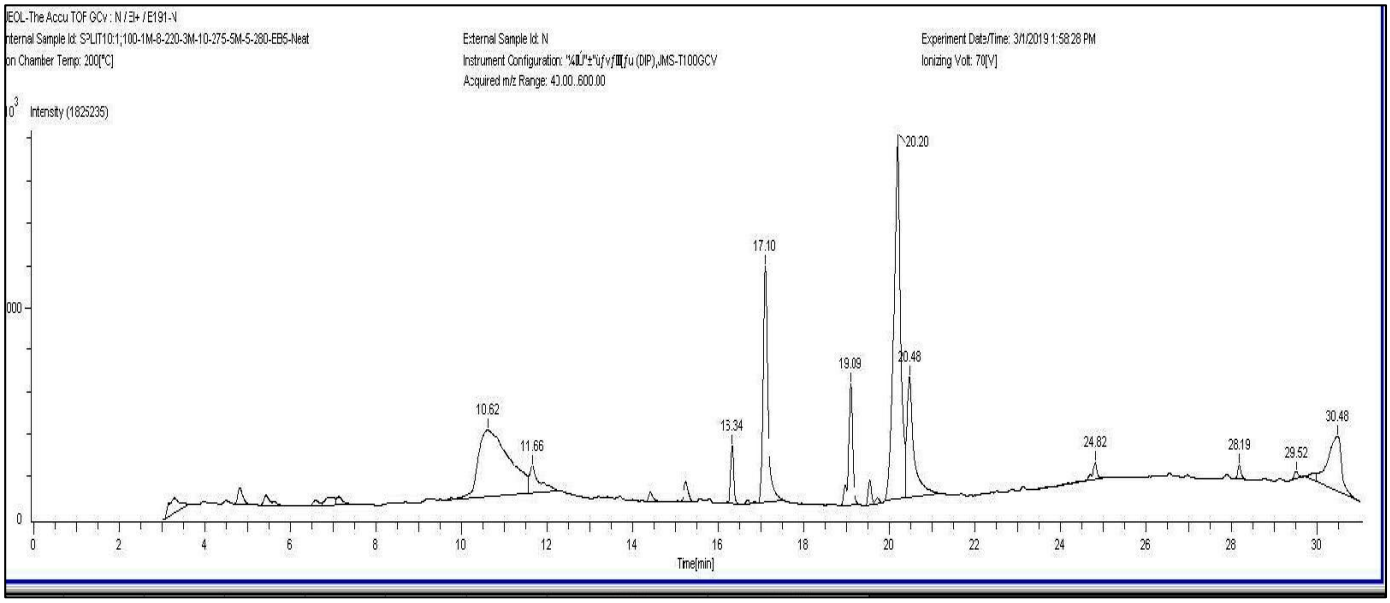

Figure No. 2 GC-HRMS Results for *P. nururi*

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Table No. 3: GC-HRMS Analysis of *Phyllanthus niruri*

Retention Time	Name of Compound	Molecular Formulae	Molecular Weight	Percent Area
29.53	9-octadecanoic acid (Z) : Phenylmethylester	$C_{25}H_{40}O_2$	372	0.68
28.19	9,12 – octadecadienoic acid (Z,Z) Phenylmethylester	$C_{25}H_{38}O_2$	370	1.28
20.40	octadecanoic acid	$C_{18}H_{36}O_2$	284	1.88
20.20	9,12,15 – octadecatrienoic acid (Z,Z,Z)	$C_{18}H_{30}O_2$	278	17.71
19.54	octadecanoic acids, methyl easter	$C_{19}H_{38}O_2$	298	57.24
19.09	9,12,15, octadecatrienoic acid, methyl ester (Z,Z,Z)	$C_{19}H_{32}O_2$	292	21.18
17.09	n – Hexa decanoic acid	$C_{16}H_{32}O_2$	256	25.59
16.23	Hexa decanoic acid, methyl easter	$C_{17}{}_{34}O_2$	270	3.94
15.26	3,7,11,15 – Tetramethyl – 2 – Hexadecen – 1 – ol	$C_{20}H_{40}O$	296	1.98
11.66	Eicosanoic acid, phenylmethyl, ester	$C_{27}H_{46}O_2$	402	5.56
10.61	1,2,3 Benzenetriol	$C_6H_6O_3$	126	43.71
4.80	1 H – Pyrrole,2,5 – dihydro – 1 – nitroso	$C_4H_6N_2O$	98	1.712

Table No. 4 : GC – HRMS compounds their Nature and Biological Activity

Sr. No	Name of Compound	Nature	Biological Activity
1	9-octadecanoic acid (Z)-Phenylmethyl ester	Fatty acid	Antiviral Activity [10]
2	9,12–octadecadienoic acid (Z,Z) Phenylmethylester	Fatty acid	Antioxidant Activity [11] Anticancer activity [12]
3	octadecanoic acid	Fatty acid	Antimicrobial Activity [13] Antiviral activity [14]
4	9,12,15 – octadecatrienoic acid (Z,Z,Z)	Fatty acid	Antibacterial Activity [15] Reduces risk of cardiovascular disease [16]
5	octadecanoic acids, methyl ester	Fatty acid	Antibacterial, Antioxidant Activity [17]
6	9,12,15, octadecatrienoic acid, methyl ester (Z,Z,Z)	Fatty acid	Antifungal and Antioxidant Activity [17]
7	n – Hexa decanoic acid	Fatty acid	Antimicrobial Activity (Rahbar, 2012)
8	Hexa decanoic acid, methyl easter	Fatty acid	Anti-Inflammatory Activity [19]
9	3,7,11,15 – Tetramethya – 2 – Hexacen – 1 – ol	Diterpene Alcohol	Antibacterial Activity [20]
10	Eicosanoic acid, phenylmethyl, ester	Dihydro Amino Acid	Not found
11	1,2,3 Benzenetriol	Fatty acid	Apoptotic [21]
12	1 H – Pyrrole,2,5 – dihydro – 1 – nitroso	Terpenoid	Not found

Diversity of decanoic acid is present in the *P. niruri*. Literature review shown that the plant harbors the maximum number of the compound that have capacity to eliminate the bacterial, fungal and viral infections

P. niruri is known for its activity for betterment of the liver functionality by modulating the enzyme profiles. As Manjrekar et al. showed the efficiency of plant extract against the ailments of liver, kidney and testes by reducing the level of malondialdehyde, alanine transaminase, aspartate transaminase, aspartate phosphatase enzyme and by increasing the level of glutathione [9]. The investigation showed the presence of expected hepatoprotective compounds which can be attributed for its role. The antiviral properties of the plant extract is because of the compounds like 9-octadecanoic acid (Z)-Phenylmethyl ester and octadecanoic acid. The plant extract have already shown its anti-HIV activity [8], the property which can be attributed to these compounds. Moreover, *P. niruri* have also shown its efficiency by possessing antimicrobial, antioxidant, anticancer, antiinflammatory, antiplasmodial, antiviral and diuretic [22]. The compounds present in this plant also gives proof for these activity.

4. Conclusion

P. niruri is most important herb mainly because of its hepatoprotective activity. In addition to this, literature shows that, plant is also known to posses antiviral properties as well as antibacterial and antifungal properties. Other potential of the plant include its capacity to fight against the cancer cells because of the presence of the compound like 1,2,3 Benzenetriol and 9,12–octadecadienoic acid (Z,Z) Phenylmethylester. Anti-inflammatory activity is shown by compunds like Hexa decanoic acid, methyl easter. 9,12,15 – octadecatrienoic acid (Z,Z,Z) also known for its property to fight against the cardiovascular diseases. Therefore this plant is under use from the ancient times for multiple ailments. This study is providing the evidences for multipurpose efficiency of *P. niruri*.

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