International Journal of Applied
and Natural Sciences (IJANS)
ISSN(P): 2319-4014; ISSN(E): 2319-4022
Vol. 8, Issue 3, Apr - May2019; 61-70

International Academy of Science,
Engineering and Technology
Connecting Researchers; Nurturing Innovations

EVALUATION OF ANTIFUNGAL ACTIVITY AND FORMULATION OF HERBAL HAIR OIL FROM *Phyllanthusniruri*

***Lavanya Prabhakar*[1]*& Mohana Priya*[2]**

[1]*Research Scholar, Department of Biotechnology, Madha Engineering College, Chennai, Tamil Nadu, India*

[2]*Research Scholar, Centre for Food Technology, Anna University, Chennai, Tamil Nadu, India*

ABSTRACT

Phyllanthusniruriis a widespread tropical herb which is well known for its medicinal properties. In the present study, we evaluated the antifungal activity of acetone, hexane, chloroform and methanolic extract of leaves of P.niruri. In addition, the formulation of herbal hair oil from P.nirurileaves against certain fungal species causing scalp disorders was also analyzed. Phytochemical screening of the extract in different solvents was carried out in order to assess the presence of terpenoids, alkaloids, flavanoids, saponin, polyphenols, and tannin. GC-MS analysis was performed to analyze the active compound present in the plant extract. The antifungal activity of the plant was investigated by using agar well diffusion method and the maximum zone of inhibition was observed in the methanolic extract. The formulated oil was also evaluated for its organoleptic properties, acid value, pH, specific gravity and density. All the parameters were found to be effective and within the standards. Altogether, the study suggests a preventive effect of Phyllanthusniruri in fungal species, but still, longer-term randomized clinical trials are necessary to confirm its therapeutic properties.

KEYWORDS: *Antifungal Activity, Extraction, GC-MS, Herbal Hair Oil, Phyllanthusniruri, Phytochemicals*

Article History

***Received:* 24 Mar 2019 | *Revised:* 29 Mar 2019 | *Accepted:* 15 Apr 2019**

INTRODUCTION

In India, many plants are mostly used as traditional medicines for the treatment of various infective diseases. Among the common medicinal plants, *Phyllanthusniruri* plays a crucial role in curing a wide range of diseases. *Phyllanthusniruri* is an herbaceous plant with an average height of 50cm. It is found all over the tropical and sub-tropical regions in south India, as well as in the Amazon rainforests, the wilds of Texas, the Bahamas, China, Peru, and other South American tropical regions. The important constituents of *P.niruri* include bioflavonoid, alkaloid, repandusinic acid, saponin, glycoside, tannin, steroid, terpenes, and lignin (hypophyllanthine and phyllanthine). The plant was also reported to contain nutrients and minerals which include carbohydrates (65.28±0.04%), crude proteins (10.50±0.15%), calcium (2209±0.50ppm) and chromium (15.23±0.13ppm).

P.niruri is widely used in the treatment of jaundice, syphilis, gonorrhea and kidney disorders. It is also known to provide relief from common health problems such as acidity, dandruff, folliculitis, and itchy skin. This plant is also known to exhibit diuretic characteristics and shows hypoglycemic results in human and animals, it is popular for kidney and liver health restoring medicine due to the presence of repandusinic acid which is an antiviral in nature. It showed improvements in results for patients with metabolic abnormalities such as hypercalciuria and hypomagnesemia. The root of the plant is

widely known for its abilities to fight against Hepatitis B and migraine. Powder made from the plant is useful in treating eye ailments as a result of liver ailments. Besides improving liver health, the plant is known for its ability to control blood glucose levels. It's bitter, diuretic, anti-inflammatory and hepatoprotective properties helps in maintaining liver health and provide relief from diabetic conditions. The leaf extract helps in the skin healing process and the fruits of the plant are useful in healing wounds, tubercular ulcers, sores, and ringworm infections.

The plant has excellent antifungal activity against *Candida albicans*, *Aspergillus species*, *Fusarium species*, and *Microsporum species*. The extracts of the plant exhibited antimicrobial activities with zones of inhibition ranging from 6 to 16mm against all the clinically important bacterial and fungal species. Maximum inhibition zone (16mm) was found in seeds against *Staphylococcus aureus* and minimum in roots (5mm) against *E.coli*. The plant species recorded a good antagonistic activity against human pathogenic microbes when compared to the standard tetracycline against selected gram-positive and gram-negative bacteria.

Hair is an epidermal derivative which is one of the vital parts increasing the overall elegance of the body. Hair fall, dandruff, split ends, grey hair are few problems involved with hair. Herbal hair oil is used in many ailments of hair.*P. niruri* promotes hair growth, hair regrowth and also provides necessary moisture to the scalp rendering beautiful hair. The present work was aimed to prepare and evaluate herbal hair oil containing herbs which are traditionally used in hair care.

The recent trend moves toward and use of natural products and herbalism. In cosmetic industries, the important source is the herbs [(1)]. Adding herbs in cosmetics is safer for our skin [(2)]. The herbal extracts mean the extract of the herbs[(3)].

MATERIALS AND METHODS

Test Microorganism

Slant cultures of *Aspergillus flavus*, *Candida albicans*, *Microsporumcanis*, *Tricodermaviridae*was obtained from Kings Institute of Preventive Medicine, Guindy, Chennai.

Plant Material

The leaves of *Phyllanthusniruri* was collected from local grounds and parks in Chennai. Then the leaves were washed, shade dried for 3 days and powdered by using an electric grinder.

Preparation of Crude Extract

15g of plant powder was extracted with 300ml of solvents (methanol, acetone, chloroform, hexane) by using Soxhlet extractor. Twelve cycles were carried out for each solvent. Finally, solvent was removed by evaporation at room temperature [(4)] polyphenols were carried by the method described by Phytochemical methods [(5)], Medicinal plants and traditional medicine in Africa [(6)],Pharmacognosy[(7)].

GC-MS Analysis

The compound present in the extract was identified by using GC-MS analysis. The retention time, peak and areas were matched with NIST library for identifying the compounds and their properties.

Determination of Antifungal Assay

Antifungal activity of the experimental plant was investigated by agar well diffusion method [8].The yeasts and saprophytic fungi were sub cultured on Sabouraud dextrose agar medium and respectively incubated at 37 degree Celsius for 24-48 hrs. Suspensions of the fungal spores were prepared in sterile distilled water. A sterile swab was dipped into the fungal suspension and was rolled on the surface of the agar medium. The plates were dried at room temperature for 15 min. Wells of required diameter were punctured in the culture media using sterilized tips. The different concentration of plant extract was administered to each well. Plates were incubated at room temperature. After incubation of 24 hours bioactivities were determined by measuring the diameter of the zone of inhibition.

Preparation of Polyherbal Hair Oil

The formula of the base contains coconut oil. Mixed leaf extracts of *Murrayakoenigii*, *Azadirachtaindica*, *Hibiscus rosasinensis*(both flower and leaf), *Eclipta prostrate*, *Phyllanthusniruri* and then added the juice of aloevera for getting a uniform paste. Into heated 500ml of virgin coconut oil, the above paste was added and boiled for 30 min on medium flame with intermittent shaking. Thereafter the oil was cooled, filtered using a muslin cloth and it was also fortified with vitamin E that helps to repair damaged hair follicles and prevents tissue corrosion, which in turn encourages hair growth preventing premature graying of hair. The oil was stored in a clean glass or plastic container for further use.

Evaluation of Herbal Hair Oil

The formulated herbal oil was evaluated for parameters like pH, acid value and organoleptic parameters. Density and specific gravity of the oil was also determined [9].

Acid Value

10ml of oil was added with 25 ml of ethanol and 25ml of ether. Phenolphthalein was used as an indicator and titrated with 0.1M potassium hydroxide solution with the formation of pale pink color as the endpoint.

Acid value= 5.61 n/w

n=Number of ml of 0.1M KOH; w=weight of oil.

Organoleptic property

Color, odor, skin irritation was determined manually. Oil was applied on hand and exposed to sunlight for 5min in order to check for any irritation.

Anti-Microbial Test

Agar well diffusion method was used for determining the antifungal activity [10]. A well was bored on a spread plate containing *Aspergillus flavus, Tricodermaviridae, Candida albicans, Microsporumcanis*and the formulated hair oil was added to it. The plates were incubated for 24-48 hr at room temperature. Zone of inhibition was measured in mm.

RESULTS

Yield Percentage of the Extract

The plant leaves were shade dried, powdered and grinded in order to obtain the extract. Thephytoconstituents were effectively fractionated using extraction solvents methanol, acetone, chloroform and hexane. Twelve cycles were carried out for each solvent and the yield percentage of the extract was calculated using the following formula and is showninTable1.

Yield= weight of dry extract x weight of dry sample × 100

Chloroform and methanol extract had a higher yield of phytoconstituents compared to acetone and hexane.

Table 1: Yield Percentage of the Extract

Solvent Used	Weight of Dry Powder(g)	Weight of Dry Extract(g)	Yield Percentage
Acetone	15	1.38	9.2
Methanol	15	3.05	20.33
Chloroform	15	4.45	22.25
Hexane	12	2.99	14.95

Phytochemical Analysis

The preliminary phytochemical analysis of acetone, chloroform, methanol andhexane extract of *Phyllanthusniruri*leaves are listed in Table 2. Methanol and acetone extracts were found to contain all phytochemicals including phenol, flavonoid, terpenoids, tannin, alkaloid, and saponins while in chloroform and hexane extract alkaloid and saponins were absent.

Table 2: Phytochemical Analysis of Extracts

Phytochemical	Acetone	Methanol	Chloroform	Hexane
Phenols	+	+	+	+
Flavonoids	+	+	+	+
Terpenoids	+	+	+	+
Tannin	+	+	+	+
Alkaloid	+	+	-	-
Saponins	+	+	-	-

'+' indicates Presence and '-' indicates Absence

GC-MS Analysis

This investigation was carried out to determine the possible phytochemical components present in *P.niruri*by GC-MS. This analysis revealed that the methanol, acetone and chloroform extracts of *P.niruri* contain various compounds having anticancer, antioxidant and hepatotropic properties. The retention time, peak area, compounds and their respective properties of methanol, acetone and chloroform extracts of *P.niruri* are shown in Table 3, Table 4 and Table 5 respectively.

Table 3: Bio Active Compounds Present in Methanolic Extract

Peak No	RT (Min)	Compound Name	Peak Area (%)
1	12.77	Patchouli alcohol	4.88
2	12.98	Patchouli alcohol	6.43
3	15.22	Cyclohexane,4-pentyl-1-(4-propylcyclohexyl)-	0.49
4	15.75	2pentadecanone,6,10,-trimethyl	15.75
5	16.05	Pentadecanoicacid,13-methyl-,methyl ester	9.39
6	16.68	Cyclopropaneoctanoic acid,2-(2pentacyclopropyl),methyl ester, trans,trams-	4.57
7	17.78	10-Octadecenoicacid, methyl ester	5.43
8	18.03	Heptadecanoicacid,9-methyl-methyl ester	11.05
9	19.02	18-nonadecenoic acid	9.27

Table 4: Bio Active Compounds Present in Acetone Extract

Peak No	RT (Min)	Compound Name	Peak Area (%)
1	11.6	Trans-4-methylthio-4-(octadecyloxy)chalone	5.97
2	12.87	Propanoicacid,2-methyl-,(decahydro-6a-hydroxy-9a-methylene-2,9-dioxouleno(4,5-b)(furan-6-yl)methyl ester	6.32
3	15.02	1-Monoclinoleoylglycerol trimethylsilyl ether	6.66
4	15.78	Fenretinide	7.32
5	16.1	Cycloprapanebutanoicacid,2,((2-((2-((2-pentacylopropyl)methyl)cyclopropyl)methyl)-methyl ester	8.20
6	16.63	Phen-1,4-diol,2,3-dimethyl-5-triflurolmethyl	10.3
7	17.87	10-octadecenoic acid,methyl ester	11.2
8	18.08	Glycine,N((3a,5a)-24-oxo)cholan-24-yl)-methyl ester	7.54
9	20.73	9-Hexadecenoic acid,9-octaenyl ester,(ZZ)	7.86
10	22.17	Cyclopropanedecanicacid,a-(acetyloxy)-2-hexyl-methyl ester	8.94
11	22.62	Acetate,10,13dimethyl-1(1-methyl-4-oxo-4-(triol-1-yl-butyl)-2,3,4,7,8,9,10,11,12,13,14,-tetradecahydro-1H-cyclopenta(a) phenanthrene-3-yl	7.51

Table 5: Bio Active Compounds Present in Chloroform Extract

Peak No	RT (Min)	Compound Name	Peak Area (%)
1	15.75	2,15-Heptadecadiene,9-(ethoxymethyl)-	9.27
2	16.05	Hexadecanoic acid, methyl ester	14.66
3	16.7	1,2-Benzenedicarboxylic acid, butyl cyclohexyl ester	9.40
4	17.78	9-Octadecenoic acid (Z)-,methyl ester	17.53
5	18.03	Cycloprapanebutanoic acid,2,((2-((2-((2-Pentacyclopropyl)methyl)cyclopropyl	9.93
6	21.3	9-Hexadecenoic acid	9.35
7	21.85	5,6-dihydroxyingal 3,7,8,12-tetraacetate	9.61
8	22.57	Phorbol 12,13.20-triacetate	9.56
9	22.87	9-hexadecenoic acid,9-octadecenyl ester,(ZZ)	10.71

Antimicrobial Activity of the Extract

The zone of inhibition of different extracts (methanol, acetone, chloroform, and hexane) against different species such as *Aspergillus flavus*, *Candida albicans* and *Trichoderma viridae* was obtained by the agar diffusion method. The zone of inhibition for different concentration of the extract against different species is shown in Table 6, Table 7 and Table 8.

Table 6: Zone of Inhibition for *aspergillus flavus*

S.No	Concentration (µg/Ml)	Methanol Extract(Cm)	Acetone Extract(Cm)	Chloroform Extract(Cm)	Hexane Extract (Cm)
1	20	0.4	0.3	0.3	-
2	40	0.6	0.5	0.6	-
3	60	0.7	0.7	0.8	-
4	80	0.9	0.9	0.9	-
5	100	1.7	1.5	1.3	-

'-' indicates no activity.

Table 7: Zone of Inhibition for *candida albicans*

S.No	Concentration (µg/Ml)	Methanol Extract(Cm)	Acetone Extract(Cm)	Chloroform Extract(Cm)	Hexane Extract
1	20	1.1	1	1	-
2	40	1.3	1.2	1.3	-
3	60	1.8	1.4	1.5	-
4	80	2.2	1.5	1.6	-
5	100	2.5	1.8	1.7	-

'-' indicates no activity.

Table 8: Zone of Inhibition For *trichoderma viridae*

S.No	Concentration (µg/Ml)	MethanolExtract(Cm)	Acetone Extract(Cm)	Chloroform Extract(Cm)	Hexane Extract
1	20	1.1	1.1	-	-
2	40	1.9	1.3	-	-
3	60	2.1	1.4	-	-
4	80	2.3	1.7	-	-
5	100	2.5	1.9	-	-

'-' indicates no activity

Antifungal activity was higher in methanol extract compared to acetone and chloroform extract. The hexane extract showed no activity against all test microorganisms such as *Aspergillus flavus*, *Candida albicans*, and *Trichoderma viridae*. The results were significant and also in correlation with the GC-MS data.The agar diffusion plates showing the zone of inhibition for different concentration of extracts in methanol, acetone, and chloroform are shown in figure 1- figure 3.

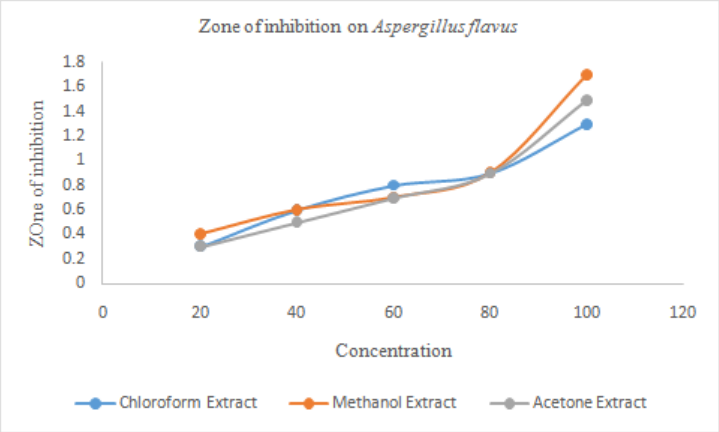

Figure 1: Antimicrobial Activity of *P. nirurion aspergillus flavus*

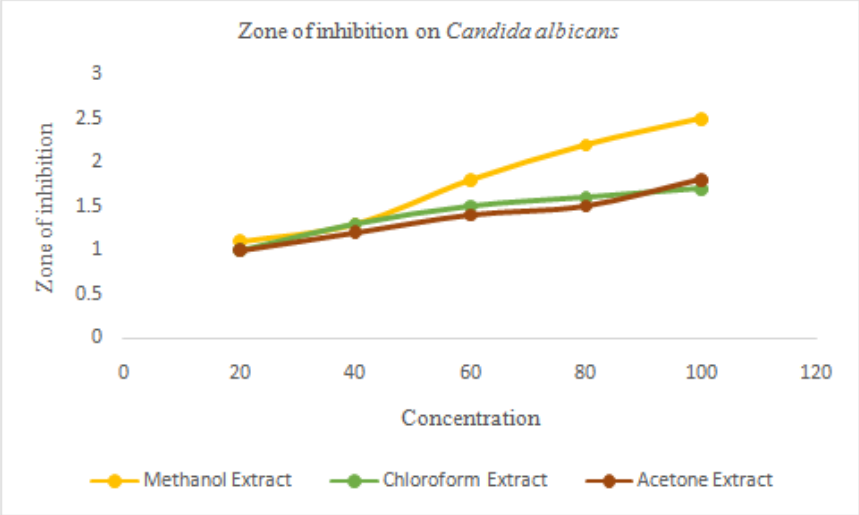

Figure 2: Antimicrobial Activity of *P. nirurion candida albicans*

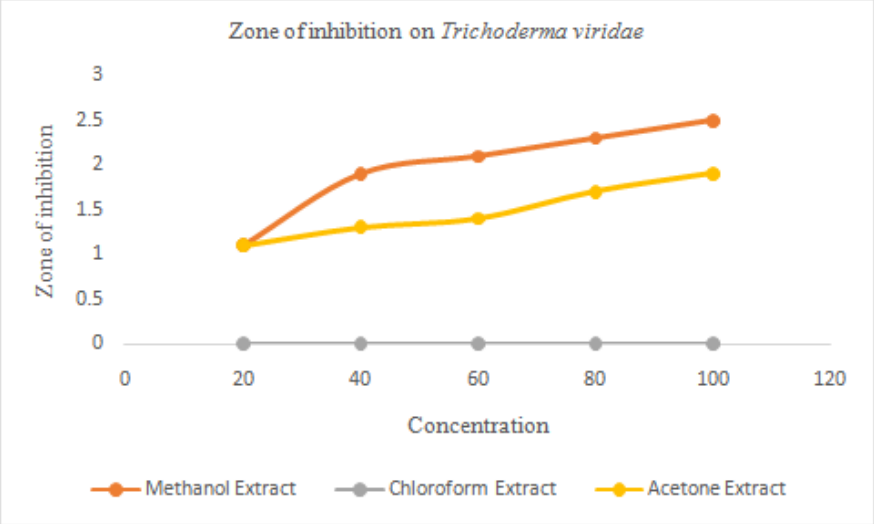

Figure 3: Antimicrobial Activity of *P. nirurion trichoderma viridae*

Herbal Hair Oil

The herbal hair oil was prepared by using coconut oil as a base [(11)].The different composition used for the preparation is shown in Table 9 and the formulated herbal hair oil is shown in figure 1.

Figure 1: Formulated Herbal Hair Oil

Table 9: Composition for Herbal Hair Oil

S. No	Ingredients	Quantity(G)
1	*Murrayakoenigii*	7.5
2	*Hibiscus rosasinensis* leaves	7.5
3	*Hibiscus rosasinensis*flowers	7.5
4	*Azadirachtaindica*	7.5
5	*Aloebarbadensis*	7.5
6	*Eclipta prostrate*	7.5
7	*Phyllanthusniruri*	15
8	Coconut oil	250 (ml)

Evaluation of Herbal Hair Oil

The prepared herbal hair oil using above mentioned ingredients was evaluated for the following parameters and the results are tabulated in Table 10.

Table 10: Evaluation of Herbal Hair Oil

S.No	Parameters	Observation
1	Color	Greenish yellow
2	Specific gravity	0.8826
3	Density	0.856
4	pH	6.1
5	Acid number	1.31
6	Irritation test	No irritation

DISCUSSION

Phyllanthusniruri is one of the common widespread plants which is well known for its medicinal properties. The leaf extract of *P.niruri* in different solvents extracted the phytochemicals at different yield percentage. Chloroform and methanol extract had a higher yield of phytoconstituents compared to acetone and hexane thereby proving the polarity based separation of phytochemicals. The presence of various phytochemicals including alkaloids, flavonoids, saponin, tannin, phenol, and terpenes were seen significantly in methanol and acetone extracts. Hence the four solvents used for extraction were found to be effective.

GC-MS analysis showed various peaks indicating the presence of components having therapeutic properties including anticancer, antimicrobial, hepatotropic and antioxidant properties. The major compounds from the methanolic extract include 8-Hexadecene,8,9-diheptyl, 2,3,9,12,16-pentamethylheptadeca-2,6,11,15-tetraene-9-carboxylic acid and 1,4-Ethanonaphthalene-6,9(4H)-dione,1,4a,5,8a-tetrahydro-4,5,710-tetramethyl-5-10-bis(methylthio)methylresponsible for antimicrobial activity of *P.niruri* which is one of the objective of our study. Similarly, the acetone extract includes Cyclopropane decanicacid,a-(acetyloxy)-2-hexyl-methyl ester, Propanoic acid, 2-methyl-(decahydro-6a-hydroxy-9a-methylene-2,9-dioxouleno(4,5-b)(furan-6-yl)methyl ester, 1-Monoclinoleoylglycerol trimethylsilyl ether andCycloprapanebutanoic acid 2,((2-((2-((2-pentacylopropyl)methyl)cyclopropyl)methyl)-methyl ester responsible for antimicrobial activity of *P.niruri*.

The major compounds of chloroform extract include Octadecenoic acid (Z)-, Methyl Ester, Hexadecanoic acid, methyl ester, 1,2-benzenedicarboxylic acid, butyl cyclohexyl Ester, Cycloprapanebutanoic acid and Hexadecenoic acid which is responsible for the antimicrobial activity of *P. niruri*.

Further, the antifungal activity was analyzed by the agar diffusion method. The extracts were found to inhibit most of the human pathogens including *Aspergillus flavus, Candida albicans*and *Trichoderma viridae.* The methanol extract was found to have higher inhibition potential compared to acetone and chloroform. However, hexane extract did not show any inhibition.

Herbal hairoil was prepared and evaluated against microbes *Aspergillus flavus, Candida albicans*and *Trichoderma viridae.* The oil was found to be devoid of irritants and did not mark any skin issues. The pH range, density, and specific gravity were found be normal. However, its activity against *Candida albicans* was found to be low.

CONCLUSIONS

Naturally, plants have medicinal properties and are used in pharmaceuticals, cosmetics, agriculture, and food industry. The study revealed that the plant has several Phytochemicals such as terpenoids, alkaloids, flavonoids, saponin, polyphenols, and tannin. Also, the plant species recorded a good antagonistic activity against human pathogenic microbes. The herbal oil prepared was found to be a safe product with its pH being in the normal range. Theacid value was also found to be satisfactory. The antimicrobial properties were sufficiently exhibited. Thus all the parameters were found to be good and within the standards. The formulated product was devoid of any potentially irritant synthetic substances like parabens as it consisted entirely of the natural substance.

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