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Phytochemical Screening of *Isodon ternifolius* and *Goniothalamus sesquipedalis* and their Antioxidant Properties

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ABSTRACT

This study focused on evaluation of antioxidant properties of methanolic extract of *Isodon ternifolius* and *Goniothalamus sesquipedalis* leaves, revealing the IC_{50} values of 34.16 and 175.59 respectively. Furthermore, these two medicinal plants are subjected for their phytochemical and elemental analysis to identify the presence of various secondary metabolites, phytoconstituents as well as elements in their extracts.

Keywords: Medicinal plants, *Isodon ternifolius*, *Goniothalamus sesquipedalis*, Antioxidant properties and Postpartum care.

INTRODUCTION

Medicinal plants are extensively used as remedies for various diseases and have shown promising potential with the efficacy of many established herbal products[1]. Plants are the natural source of organic and inorganic components. The organic components are the mainly bioactive in nature, whereas the minor part is an inorganic component found in the range of 1ug/1g, called trace elements[2-3]. Electrolytes like Na, Mg, K, and Ca are essential for basic life functioning and the trace elements facilitate vital biological reactions as cofactor or catalyst for various enzymes. Thus, the imbalance of these metals lead to various health issues[4-5]. Manipur of North-Eastern India belongs to the Indo-Burma biodiversity hotspot, flourishing with rich floral and faunal species diversity[6]. Of the various medicinal plants found in Manipur, we were intrigued to examine the biological and phytochemical screening of two plants *I. ternifolius* Kudo and *G. sesquipedalis* Hooker f. and Thomas, in order to substantiate biologically their use in folk medicine.

I. ternifolius is a perennial herb belonging to family Lamiaceace, with its leaves verticillate in nature, and its stem has six edges rather than the four regular edges. The plant has been commonly used in folk medicine as the antidote for smallpox, to treat skin disease, and to prepare hair lotion (chenghi in Manipuri)[7]. Besides, it is well known for the Chinese anti-hepatitis drug "fu fang san ye xiang cha cai pian" used to treat chronic and acute hepatitis and hepatitis B[8]. Also, diterpenoid[9-10], ternifolipyrons A-J[11], lignan and phenylethanoid compounds[12] were known to be isolated from it. Previous studies have shown that it possesses anti-inflammatory and icterohepatitis properties[13].

On the other hand, *G. sesquipedalis* of Indian origin[14] belonging to the family Annonaceae is an important medicinal plant. The leaves are oval shaped with sharp ends and the flowers with greenish or yellowish in color and are solitary and axillary. The decoction of fresh leaf is used as remedy for stomach pain[7]. It is also known to exhibit cytotoxic, abortifacient, antitumor, pesticidal, teratogenic and embryotoxic activities and bioactive compounds like goniopedaline, aristololactam A-II, taliscanine, etc were present in the *G. sesquipedalis*[15-16].

Moreover, smoke from burnt leaves of these plants is used for postpartum care as well as traditional fumigation to treat small pox, pustule and ulcer[17]. So far, there is limited studies on *G. sesquipedalis*, other than anthelmintic and insecticidal[18], antibacterial[19], as well as antimicrobial activities[20] etc. Similarly, the anti-inflammatory[12], cytotoxic and anticancer activities[21,12] of *I. ternifolius* are some reported biological activities. However, no studies have been carried out for trace elements analysis and comparative studies of both the plants. Thus, in continuation of our interest in medicinal plants found in Indo-Burma biodiversity hub[22-24], we are reporting herewith the phytochemical analysis, elemental analysis and the antioxidant activities of these two plants.

MATERIALS AND METHODOLOGY

Collection and Authentication of Plant Material

I. trenifolius and *G. sesquipedalis* plants were freshly collected during August/September 2022 from different parts of Thoubal district, Manipur (India). The plants were botanically identified and deposited (with voucher no. 001228, MUMP and 001227, MUMP) in the Department of Life Science, Manipur University, Canchipur-795003, Manipur. The leaves of the plants were dried and grinded into powder form to determine the phytochemical and elemental analysis and antioxidant properties. The powder formed was stored in a closed container until used.

Chemicals and materials

The chemicals used were all analytical grade reagents. The chemicals 2,-2-diphenyl-1-picrylhydrazyl (DPPH) (Merck, India), Ninhydrin's solution, Molish's reagent (Alfa Aesar, UK), Millon's and Benedict's reagents (Merck, USA) were supplied by Eastern Equipments, Imphal-795001, India.

Preparation of plant extracts

The grounded leaves of these plants (200 g) was soaked in 1000 mL each of aqueous ethanol (8:2, v/v) and aqueous methanol (8:2, v/v) separately at 23-25°C for 74 h with occasional stirring. Then, after every 24 h the extracts were collected by decantation and required solvent was added to the residue. The collected extracts were concentrated and were kept at 4°C for further experiment.

Phytochemical analysis of the extracts

Soxhlet technique was carried out to perform phytochemical analysis by taking 25 gof leaves powder. Different solvents (ethanol, chloroform, ethyl acetate, and methanol) were separately extracted. The collected extracts were again kept at 4°C to perform the experiments. Then, 5 g of the plant powder and 250 mL of distilled water were mixed in a conical flask and heated on a heating mantle at 35-42 ^{O}C with continuous stirring for 25 minutes. The resultant aqueous mixture was filtered with an appropriate filter paper and the filtrate was stocked for further experiments.

Qualitative analysis of the extracts

The aqueous, methanolic, ethyl acetate, chloroform and petroleum ether extracts screening was carried out to establish the occurrence of the phytoconstituents by following the reported procedures[25-26].

Tests for proteins

Millon's test: When the crude extract was mixed with Millon's reagent (2 mL), on gentle heating white precipitate appeared. Which then

turns into red color indicating the presence of protein in the test sample.

Tests for carbohydrates

Fehling's solutions test: Equal quantity of Fehling solutions A and B were boiled and add to the extracts. The formation of red precipitate confirmed the occurrence of sugars (reducing).

Benedict's reagent test: Benedict's reagent (3 mL) was boiled with the extracts. The appearance of reddish-brown confirmed for carbohydrates in the extracts.

Iodine test: When the extract was added by iodine solution (2.5 mL) and prevalence of purple or dark blue solution proved the occurrence of carbohydrate in the extracts.

Test for phenols

To the extracts, few drops of 1 mL of 1% ferric chloride and 1 mL of potassium ferrocyanide were added. The formation of blue-green color indicated the presence of phenols.

Test for tannins

Addition of 1% $FeCl_3$ solution were added to the test extracts. The appearance of brownish-green or bluish-black coloration indicated the occurrence of tannins.

Tests for flavonoids

When 1.5 mL of 2% NaOH solution was added to the plant crude extracts, if yellow color appears, and addition of 3 drops of dil. HCl makes colorless, it confirms for flavonoids in the sample.

Test for saponins

To the plant extracts, addition of 5 mL of distilled water with vigorous shaking, the development of foam showed the existence of saponins.

Tests for glycosides

Liebermann's test: The mixture of 3 mL of acetic acid and 3 mL of chloroform was added to the plant extracts. To this mixture few drops of concentrated H_2SO_4 was added, green color showed the presence of aglycone steroidal portion of glycosides.

Test for steroids

To the mixture of 2 mL of $CHCl_3$ and conc. H_2SO_4, the plant extracts were added. The appearance of red coloration in the $CHCl_3$ layer indicated the occurrence of steroids.

Test for terpenoids

To the plant extracts, 2 mL of $CHCl_3$ was added and then boiled with 2 mL of conc. H_2SO_4. The formation of grey color mass showed the presence of terpenoids.

Elemental analysis

The elemental analysis was determined using the standard calibration curve method[27-28]. Each plant materials (0.20 g) were taken in 100 mL flask and 6.0 mL of mixed acid comprising of HNO_3, H_2SO_4 and $HClO_4$ in the ratio of 5:1:0.5 (mL). The resulting mixture after following specified procedure was filtered and the filtrates were examined for the qualitative analysis of different elements using atomic absorption spectroscopy (AAS). The AAS (model AA-7000F) unit with deuterium-arc background correction was used for determination of elements Ca, Fe, Mn, Cu, Ni, Zn and Pb up to ppm (ug/L).

Inductively coupled plasma mass spectrometer (ICP-MS) (Agilent Technologies, Model: 7900) was used for elements analysis like Li, Na, Mg, K, Ca, Fe, Cu, Zn, As and Pb up to ppb (ug/g). The relative standard deviation (%RSD) = (SD/mean)x100, where mean is provided in ppm or ppb). Here, RSD values of ICP-MS techniques were 2-5 % and 5-10%, respectively.

Antioxidants properties

Preparation of methanolic plant extracts

About 20 g of the grinded plants powder were soaked in 1.5 L of methanol and extracted by using Soxhlet. The extraction was done about 12 h and then the extracts were evaporated by using rotavapor. The resultant crude extract was saved at $4^{O}C$ until antioxidant test was done.

Preparation of working plants extracts and standard ascorbic acid solution

A standard solution of a conc. of 1 mg/mL, 0.5/mL and 2 mg/mL in methanol were firstly ready for the ascorbic acid, *I. ternifolius* and *G. sesquipedalis* respectively. The working solutions

were prepared by changing the concentrations of standards and plants in the vol. of 20, 40, 60, 80 and 100 µL by successive dilution with the mentioned solvent from the standard solution.

DPPH antioxidant activity

The antioxidant properties of these plants was carried out using DPPH antioxidant assay with minor adjustments in the procedures[29].

1 mL of test solution of methanol dissolving in 1 mL of DPPH solution (0.1 mM) was measured for increase in DPPH absorbance after 20 min of incubation at 25^{O}C at 517nm. Ascorbic acid (1 mM), showing maximum absorbance at 90.36±1.05 µg/mL was considered as a reference solution in this assay.

DPPH inhibition activity (%) = (A-B)/A ×100%

A = optical density (blank),

B = optical density (sample).

RESULTS AND DISCUSSIONS

The AAS showed that major elements such as Fe, Ca, Mn, Cu, Ni, Zn and Pb have been found in both the plants. However, Fe (7.22 ppm) is found in more concentration in *I. ternifolius* and Ca (15.18 ppm) in *G. sesquipedalis*. The ICP-MS data obtained are also presented in Table 2, showing that both the plants exhibit the maximum concentration of the elements Na, K, Ca, Mg, Fe, Cu and Zn. Among of them, K and Mg are found as the maximum highest concentration in both the plants. The low concentration of Li, As and Pb was found in both the plants but Pb was found to be absent in *I. ternifolius*. The high concentration of certain metals, such as Mg^{+2} and K^{+} may be attributed for their appropriate development and regular functioning of the plant. On the other hand, metals such as Li, As and Pb are present in trace amounts which are non-essential to human body and toxic so it might be used as disinfectants and for the inhibition of pests, insects etc.

The result of the phytochemical screening tests of both the plants showed the occurrence of phytochemical constituents such as alkaloids, cardiac glycosides, reducing sugars, phenols and flavonoids as presented in Table 2. The glycosides are present in all extracts except petroleum ether.

Table 1: Elemental analysis resulted by AAS and ICP-MS

Element	*I. ternifolius*		*G. sesquipedalis*	
	AAS (ppm)	ICP-MS Ug/g or ppb	AAS (ppm)	ICP-MS Ug/g or ppb
Li		0.14		0.14
Na		33.33		66.37
K		18568.37		13606.85
Mg		5102.03		5526.03
Ca	5.78	169.31	15.18	500.28
Fe	7.22	601.65	4.02	522.21
Cu	0.05	3.74	0.04	3.46
Ni	0.05		0.19	
Zn	0.39	15.57	0.38	11.16
Mn	1.02		4.79	
As		0.065		0.02
Pb	0.40	0	0.33	0.13

Table 2: Phytochemical screening tests for the petroleum ether (PE), $CHCl_3$, ethyl acetate (EA), methanol, aqueous solutions of *I. ternifolius* and *G. sesqupedalis* extracts

	Extract	Alkaloids	Proteins	Carbohydrates	Phenols	Tannins	Flavonoids	Saponins	Glycosides	Steroids	Terpenoids
I. ternifolius	PE	+	-	+	-	-	+	+	-	+	+
	$CHCl_3$		-	+	-	-	+	+	+	+	+
	EA	+	-	+	-	-	+	+	+	+	+
	CH_3OH	+	-	-	+	+	+	+	+	-	-
	H_2O	+	-	-	+	-	+	+	+	-	-
G. sesquipedalis	PE	+	-	+	-		+	+	-	+	+
	$CHCl_3$			+	+		+	+	+	-	-
	EA	+	-	+	+		+	+	+	-	-
	CH_3OH	+	-	+	-		+	+	+	-	-
	H_2O	+	-	+	-		-	+	+	-	-

(+) indicates the presence of phytochemicals and (-) indicates the absence of phytochemicals. Blank for no experiment conducted

Antioxidant activity using Ascorbic acid as standard equivalent

In order to study about the antioxidant's properties of the two methanolic extracts, the two extracts were investigated with the help of DPPH method using ascorbic acid as the standard

reference. Depending upon the scavenging property of the plants, the concentrations were made ranging from 10-50 µg/mL for *I. ternifolius* and 40-200 µg/mL for *G. sesquipedalis* respectively. In our study, "o" inhibition was taken for DPPH only. The findings are presented in the Table 3 and explained with the help of portraying graph as shown in Figure 1.

Table 3: Percentage inhibition activity for reference sample, *I. ternifolius* and *G. sesquipedalis* extracts

Ascorbic acid			*I. ternifolius*			*G. sesquipedalis*		
Vol. of the sol.	Conc.	%inhibition	Vol. of the sol.	Conc.	%inhibition	Vol. of the sol.	Conc.	%inhibition
20	2	0.8425	20	10	0.8855	20	40	0.8645
40	4	0.731	40	20	0.7165	40	80	0.7175
60	6	0.6145	60	30	0.5505	60	120	0.6085
80	8	0.477	80	40	0.3705	80	160	0.5355
100	10	0.3485	100	50	0.217	100	200	0.4135

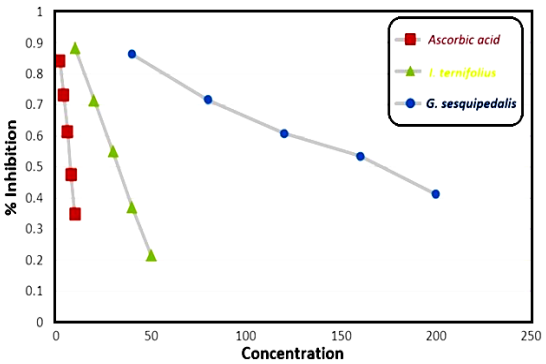

Fig. 1. Inhibition activity of ascorbic acid, *I. ternifolius* and *G. sesquipedalis* extracts

From the above data, the calculated IC_{50} for *I. ternifolius* and *G. sesquipedalis* are 34.16 and 175.59 respectively, w.r.t the standard IC_{50} of ascorbic acid.

CONCLUSION

The phytochemical screening of extracts showed that glycosides, alkaloids, steroids, carbohydrates and saponins are found in most of the extracts as preliminary investigation. Besides, the two plants have been observed the presence of Ca, Mg, Ni, Zn and Cu within the permissible level set by WHO/ FHO. The findings will be of tremendous benefits for the formulations of new herbal drugs along with various combinations. Besides, the DPPH studies revealed that the two plants have effective antioxidant activities and further studies are needed to ascertain their medicinal properties.

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Conflicts of interest

We have no competing interests that could influence our research or findings.

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