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LC- HRMS and Phytochemicals Analysis of *Gnidia glauca* L. leaf crude extract with different solvents

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Abstract

The member of the Thymelaeaceae family, *Gnidia glauca* (Fresen) Gilg, has been found to possess a multitude of traditional phytomedicinal and agrochemical applications. When viewed in its entirety, *Gnidia glauca* L. (Thymelaeaceae) stands as the most extensive genus within the family, boasting a remarkable 140 to 160 species scattered across Africa, Arabia, India, Madagascar, and Sri Lanka. The leaves of *Gnidia glauca* L., which are meticulously extracted using chloroform, methanol, and alcohol, yield a diverse array of chemically intricate and biologically potent substances. A staggering number of over 90 compounds have been successfully identified within it. The meticulous phytochemical analysis of *Gnidia glauca* leaves has revealed the presence of alkaloids, phenols, flavonoids, tannins, glycosides, saponins, terpenoids, and steroids. The objective of this endeavor is to employ LC-HRMS-based techniques to meticulously analyze and detect both targeted and non-targeted phenolic compounds derived from the leaves of *Gnidia glauca* L.

Keywords: *Gnidia glauca* L, Thymelaeaceae, extraction, phytochemical profile, LC-HRMS.

Introduction

Gnidia glauca L. is an indigenous botanical specimen possessing a rather intricate chemical composition encompassing alkaloids, flavonoids, saponins, steroids, organic acids, polysaccharides, and diverse trace elements. These compounds exhibit advantageous properties such as anti-inflammatory effects, pain alleviation, inhibition of tumor cell proliferation, and more. Owing to the deleterious side effects and health hazards associated with chemically synthesized medications, the emergence of complementary and alternative medicine has transpired. Medicinal plants have garnered significant attention due to their biocompatibility and efficacy. *Gnidia glauca* L. holds a prominent position in the realm of mythology, traditional phytomedicine, and agrochemical applications worldwide.

In addition to its utilization in the treatment of tumors, wounds, snake bites, sore throats, and burns, *Gnidia glauca* L. is renowned for its piscicidal, insecticidal, molluscidal, and even homicidal properties when employed as arrow poisons. Furthermore, its antineoplastic activity is purportedly remarkably superior (17). Phytochemical investigations of *Gnidia glauca* L. have unveiled the presence of toxic diterpene esters of the daphnane variety, which represent the principal orthoesters found in plants and exhibit remarkable biological activities, including antineoplastic and cytotoxic effects. *Gnidia glauca* L. is abundant in diterpene esters, coumarins, flavonoids, chromones, lignans, and neolignans (3). These chemical

constituents of *Gnidia glauca* L. hold potential as foundational materials for the synthesis of analogues capable of treating a diverse array of ailments.

Materials and Methods

Collection and identification of plant

Gnidia glauca (Fresen) Gilg was collected from Nivkane village of Patan, Maharashtra. The plant was authenticated from Dept. of Botany, Shivaji University Kolhapur and voucher specimens (VSN/ 001) were deposited in the department for future reference.

Extraction of plant material using solvents

The leaves of *Gnidia glauca* (Fresen) Gilg were thoroughly cleansed on four to five occasions with flowing water from the faucet and once with sterile water prior to being dried in the shade, pulverized, and utilized for extraction. A quantity of 20 grams of powdered plant material underwent a rigorous 48-hour (1:10) soxhlet extraction employing the solvents methanol, chloroform, and alcohol. Subsequently, the extracts were filtered through Whatman filter paper No. 1 and dried in a water bath shaker. In order to ascertain the presence of various secondary metabolites, all of the extracts were subjected to preliminary phytochemical screening and LC-HRMS analysis (24).

Preliminary analysis of *Gnidia glauca L.* leaf extracts

The concentrates were subjectively tested and assessed for the existence of various phytochemical constituents employing standard methodologies to identify the constituents as delineated by Harborne (9).

Test for alkaloids

Dragondroff's test **–** Crude extract was mixed with Dragondroff's reagent (potassium bismuth iodide solution). Reddish brown precipitate was formed which suggested the presence of alkaloids. 5 ml of the extract was added to 2 ml of HCl. To this acidic medium, 1 ml of Wagner's reagent was added. A reddish precipitate brown produced immediately indicates the presence of alkaloids.

Test for flavonoids

1.5 ml of a 50% methanol solution was used to treat 4 ml of the extract solution. Metal magnesium was added after the solution was warmed up. 5 to 6 drops of concentrated hydrochloric acid were added to this solution, and flavonoids with a red color and flavones with an orange color were observed. 2ml of extract solution add with 2 ml of diluted 10% sodium hydroxide (NaOH), golden yellow precipitate shows presences of flavonoids.

Test for glycosides

1 ml of Fehling's solution was heated and added to a small amount of extract. An orange precipitate indicates the presence of glycosides.

Test for tannins

Ferric chloride reagent added to the filtrate. The presence of tannins was confirmed by a precipitate that was green, blue-green, or blue-black. 2 to 3ml of extract solution and add 10% ferric chloride reagent green, blue-green, or blue-black precipitate occur it shows presences of tannins

Test for saponins

The saponins were found using the frothing test. In a water-filled test tube, 0.5 grams of extract from each part was taken. The presence of saponins is indicated by the mass of bubbles produced when the solutions were warmed up. Foam test – 2ml of extract solution and add 20 ml of distilled water and shake vigorously wait for 3 min formation of honeycomb like froth shows the presences of saponins.

Test for quinones

To a modest quantity of extract solution, sulphuric acid is added. Quinones are identified

by their red appearance.

Test for terpenoids

2 ml of chloroform was mixed with 5 ml of each extract. To make a layer, 3 ml of concentrated H_2SO_4 was added. Terpenoids were detected by the formation of a reddish-brown precipitate at the interface.

Test for phlobatannins

When each plant sample's aqueous extract was boiled in 1% aqueous hydrochloric acid, a red precipitate fell as evidence for the Phlobatannins.

Test for steroid

Chloroform and 2.5 ml of acetic anhydride were used to treat the extract. After that, a concentrated solution of sulphuric acid was slowly added, and the terpenoids' reddish-violet color and the steroids' greenish-blue color were observed. Then 2 ml extract solution dissolved in 2 ml of chloroform and add concentrated H_2SO_4 side by side carefully red precipitate shows that steroid is present. 2 ml of extract solution then add 1 ml of ethyl acetate and mix into 2 ml of chloroform and few drops of concentrated H_2SO_4 side by side carefully red precipitate shows that steroid is present.

Liquid chromatography high resolution mass spectrometry (LC-HRMS) characterization

The determination of bioactive compounds in the leaf extracts was conducted at the esteemed Center for Applications in Mass Spectrometry (CAMS), Venture Laboratory, located in Pune. This analysis was performed utilizing the sophisticated LC-HR-MS technique (Liquid Chromatography-High Resolution-Mass Spectrometry). Prior to analysis, the methanolic, chloroformic, and alcoholic extracts underwent centrifugation at a speed of 12,000 revolutions per minute for duration of 10 minutes. The HPLC system employed consisted of two pumps and an automated injector. The separation process was accomplished using an Agilent Q-ToF G6540B instrument, which was connected to an Agilent 1260 Infinity II HPLC system equipped with an Agilent Eclipse XDB-C18 column measuring 3X150 mm and possessing a particle size of 3.5 microns. Two distinct mobile phases were employed: A-0.1% formic acid in water and B-0.1% formic acid in acetonitrile, with a flow rate of 0.3 mL/min. The LC conditions were as follows: an initial concentration of 5% in phase B from 0 to 3 minutes, followed by a linear increase from 5% to 95% between 2 and 25 minutes, maintaining 5% to 95% from 25 to 28 minutes, and transitioning from 5% to 95% between 25 and 28.1 minutes. Finally, the concentration returned to 0% to 5% during the interval of 28.1 to 30 minutes. For the MS analysis, the Dual AJS ESI Mass spectrometer was employed in positive ionization mode, utilizing data-dependent automatic switching between MS and MS/MS acquisition modes.

Results and Discussion

Table No. 1.Qualitative phytochemical analysis of *Gnidia glauca* leaf in various solvents.

Sr. No	Metabolites	*Gnidia glauca* leaves		
		Methanol	Alcohol	Chloroform
1	Alkaloid	+ve	+ve	+ve
2	Flavonoids	+ve	+ve	+ve
3	Glycosides	-ve	+ve	-ve
4	Tannins	+ve	+ve	-ve

5	Saponins	+ve	+ve	+ve
6	Quinones	+ve	+ve	-ve
7	Terpenoids	+ve	-ve	+ve
8	Phlobatannins	-ve	-ve	-ve
9	Steroid	+ve	+ve	+ve

Note – (+ve) present, (-ve) absent

Table 2. Phytochemicals screening of solvent (Alcohol) extracts of *Gnidia glauca* leaves by LC-HRMS

Sr. No	Compound Name	Regulator / Inhibitor	Chemical formula	Rt. Time	Mass	Score	Importance
1	3-Phenylpropyl glucosinolate	Inhibitor	$C_{16}H_{23}NO_9S_2$	2.356	437.0802	91.60	Antimicrobial activity, in vivo cytotoxicity
2	Zwittermicin A	Inhibitor	$C_{13}H_{28}N_6O_8$	3.805	396.1969	97.46	Suppress plant disease
3	Famciclovir	Inhibitor	$C_{14}H_{19}N_5O_4$	3.902	321.1427	93.53	Herpes virus
4	Dimepiperate	Inhibitor	$C_{15}H_{21}NOS$	3.973	263.1353	90.68	Moderately hazardous class II (Herbicide)
5	Pyranodelphinin A	Inhibitor	$C_{30}H_{33}O_{16}$	7.388	649.1774	98.12	Natural fungicide
6	N-heptanoyl-homoserine lactone	Regulator/ Inhibitor	$C_{11}H_{19}NO_3$	10.327	213.1355	94.18	Regulation of virulence, infection prevention, and septicemia in fish.
7	Erinapyrone C	Inhibitor/ regulator	$C_8H_{10}O_5$	12.215	186.0537	92.41	Cytotoxicity of HeLa cells and nerve growth
8	Kiwiionoside	Inhibitor	$C_{19}H_{34}O_9$	12.393	406.2202	97.00	Antirepellent compound
10	(+)-Gallocatechin	Inhibitor	$C_{15}H_{14}O_7$	14.061	306.0742	98.96	Insecticidal properties
11	Ginkgolide C	Inhibitor	$C_{20}H_{24}O_{11}$	14.333	440.1316	99.67	Pesticidal properties
12	Garcimangosone D	Inhibitor	$C_{19}H_{20}O_9$	14.701	392.1105	98.19	Pesticidal properties
13	Khellolglucoside	Inhibitor	$C_{19}H_{20}O_{10}$	16.390	408.1058	92.61	Pesticidal properties
14	6-Undecanone	Inhibitor	$C_{12}H_{22}O$	16.508	170.1678	96.28	Pesticidal properties

15	Lansiuma-mide C	Inhibitor	$C_{18}H_{19}NO$	17.076	265.1471	96.32	Pesticidal properties
16	3R-hy-droxy-oct-adecanoic acid	Inhibitor	$C_{18}H_{36}O_3$	25.019	300.2663	98.63	Antioxidant, hypo-cholesterolemic, nematicide, pesticide, 31antiandrogenic, flavour, hemolytic, 5-alpha reductase inhibitor
17	10-Hydroxy-myristic acid methyl	Inhibitor	$C_{15}H_{30}O_3$	24.558	258.2196	95.6	Antioxidant, hyper-cholesterolemic,-cancer-preventive, cosmetic, nematicid.
18	Cyclic de-hy-poxanthine futalosine	Inhibitor	$C_{14}H_{14}O_7$	17.499	294.0744	98.18	Enzymatic proper-ties. Efficient growth of the *Streptomyces coelicolor* mutant

Table No. 3. Phytochemicals screening of solvent (Methanol) extracts of *Gnidia glauca* leaves by LC-HRMS

Sr. No	Compound Name	Regulator / Inhibitor	Chemical formula	Rt. Time	Mass	Score	Importance
1	N'-Hydroxyneosaxi-toxin	Inhibitor	$C_{10}H_{17}N_7O_6$	3.392	331.1248	92.52	Paralysis the nerve cells
2	Mycinamicin IV	Inhibitor	$C_{37}H_{61}NO_{11}$	15.932	695.4258	90.43	Anticancer
3	Lycoperoside D	Inhibitor	$C_{39}H_{65}NO_{12}$	16.182	739.4513	94.53	Inflammatory diseases, Skin health
4	Aloesol 7-glucoside	Inhibitor	$C_{19}H_{24}O_9$	17.265	396.1415	93.68	Inflammation and ulcer
5	Tiamulin	Inhibitor	$C_{28}H_{47}NO_4S$	20.119	493.3241	92.50	Antibacterial drug
6	3-Feruloyl-1,5- quino-lactone	Inhibitor	$C_{17}H_{18}O_8$	20.071	350.1001	98.48	Biomass deg-radation
7	4,4-Difluoro-pregn-5-ene-3,20- di-one	Inhibitor	$C_{21}H_{28}F_2O_2$	20.889	350.2046	93.53	induced aller-gic rhetis
8	Nicotine imine	Regulator	$C_{10}H_{13}N_2$	28.037	161.1081	92.98	Biomarker for oral cancer pa-tient
9	16-hydroxy hexadeca-noic acid	Regulator	$C_{16}H_{32}O_3$	24.538	272.2360	93.07	steriodal estro-gen

10	Prasterone sulfate	Inhibitor	$C_{19}H_{28}O_5S$	3.260	368.1658	96.10	It is a universal precursor for the peripheral local production and action of estrogens and androgens in target tissues such as brain, bone, skin, and adipose tissue.
11	Zwittermicin A	Inhibitor	$C_{13}H_{28}N_6O_8$	3.805	396.1969	97.46	Suppress plant disease
12	Famciclovir	Inhibitor	$C_{14}H_{19}N_5O_4$	3.902	321.1427	93.53	Herpes virus
13	Decarbamoylneosaxitoxin	Regulator	$C_9H_{16}N_6O_4$	9.866	272.1244	93.73	Neurotoxin
14	Musca-aurin-VII	Regulator	$C_{15}H_{17}N_4O_6$	10.989	349.1141	97.25	contribute to the pigment pattern of fly agarics
15	Hydralazine	Inhibitor	$C_8H_8N_4$	11.258	160.0750	98.18	Therapy for hypertension
16	Kiwiionoside	Inhibitor	$C_{19}H_{34}O_9$	12.393	406.2202	97.00	Anti-repellent compound
17	(+)-Gallocatechin	Inhibitor	$C_{15}H_{14}O_7$	14.061	306.0742	98.96	Insecticidal
18	Ginkgolide C	Inhibitor	$C_{20}H_{24}O_{11}$	14.333	440.1316	99.67	Pesticidal
19	Garcimangosone D	Inhibitor	$C_{19}H_{20}O_9$	14.701	392.1105	98.19	Pesticidal
20	Khellolglucoside	Inhibitor	$C_{19}H_{20}O_{10}$	16.390	408.1058	92.61	Pesticidal
21	10,16-dihydroxy-palmitic acid	Inhibitor	$C_{16}H_{32}O_4$	21.932	288.2301	96.10	Antioxidant, hypocholesterolemic, nematicide, pesticide, antiandrogenic, flavor, hemolytic, 5-alpha reductase inhibitor
22	Cyclic de-hypoxanthine futalosine	Inhibitor	$C_{14}H_{14}O_7$	17.499	294.0744	98.18	Enzymatic properties. Efficient growth of the *Streptomyces coelicolor* mutant

LC- HRMS and Phytochemicals analysis of *Gnidia glauca* L. leaf crude extract with different solvents

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Table No.4 Phytochemicals screening of solvent (Chloroform) extracts of *Gnidia glauca* leaves by LC-HRMS

Sr. No	Compound Name	Regulator / Inhibitor	Chemical formula	Rt. Time	Mass	Score	Importance
1	cortisol 21-sulfate	Regulator	$C_{21}H_{30}O_8S$	3.137	442.1657	97.17	Affects behavior through its direct action on the central nervous system, its effects on intermediary metabolism or negative feedback on pituitary ACfH release.
2	ACRL Toxin II	Inhibitor	$C_{17}H_{24}O_5$	17.328	308.1629	96.05	host specific pathotoxic, plant and animal cell death
3	Mycinamicin IV	Inhibitor	$C_{37}H_{61}NO_{11}$	15.932	695.4258	90.43	Anticancer
4	Pitheduloside A	Inhibitor	$C_{41}H_{66}O_{13}$	16.434	766.4512	94.91	Larvicidal and ovicidal activity
5	Tiamulin	Inhibitor	$C_{28}H_{47}NO_4S$	20.119	493.3241	92.50	Antibacterial drug
6	Nicotine imine	Regulator	$C_{10}H_{13}N_2$	28.037	161.1081	92.98	Biomarker for oral cancer patient
7	16-hydroxy hexadecanoic acid	Regulator	$C_{16}H_{32}O_3$	24.538	272.2360	93.07	steriodal estrogen
8	3 Irinotecan	Inhibitor	$C_{33}H_{38}N_4O_6$	28.415	586.2772	93.40	Cancer treatment
9	Famciclovir	Inhibitor	$C_{14}H_{19}N_5O_4$	3.902	321.1427	93.53	Herpes virus
10	(+)-Gallocatechin	Inhibitor	$C_{15}H_{14}O_7$	14.061	306.0742	98.96	Insecticidal
11	Garcimangosone D	Inhibitor	$C_{19}H_{20}O_9$	14.701	392.1105	98.19	Pesticidal
12	3R-hydroxy-octadecanoic acid	Inhibitor	$C_{18}H_{36}O_3$	25.019	300.2663	98.63	Antioxidant, hypocholesterolemic, nematicide, pesticide, antiandrogenic, flavour, hemolytic, 5-alpha reductase inhibitor
13	3-Hydroxyindolin-2-one	Inhibitor	$C_8H_7NO_2$	5.161	149.0474	98.00	Allergenic, anesthetic, antibacterial, anticancer, antimutagenic, antipeptic, antiseptic, antispasmodic, antitumor, candidicide, flavour, insecticide, nematicide, pesticide, sedative, termiticide, tyrosinase inhibitor
14	1-(m - Methoxycinnamoyl) pyrrolidine	Inhibitor	$C_{14}H_{17}NO_2$	13.110	231.1249	94.64	Allergenic, anesthetic, antiodontalgic, antipruritic, antiseptic, flavour, fungicide, pesticide, sedative.

15	Cyclic de-hypox-anthine futalosine	Inhibitor	$C_{14}H_{14}O_7$	17.499	294.0744	98.18	Enzymatic properties. Efficient growth of the *Streptomyces coelicolor* mutant
16	Proglumide	Inhibitor	$C_{18}H_{26}N_2O_4$	16.767	334.1885	91.98	Inhibited gastrin-stimulated growth. (Treatment of stomach ulcers)

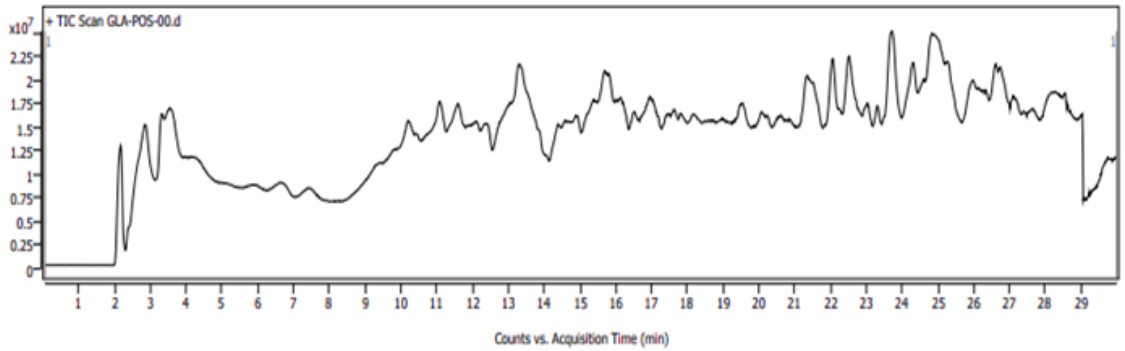

Fig. 1. LC-HRMS of *Gnidia glauca* Leaves Alcohol extract

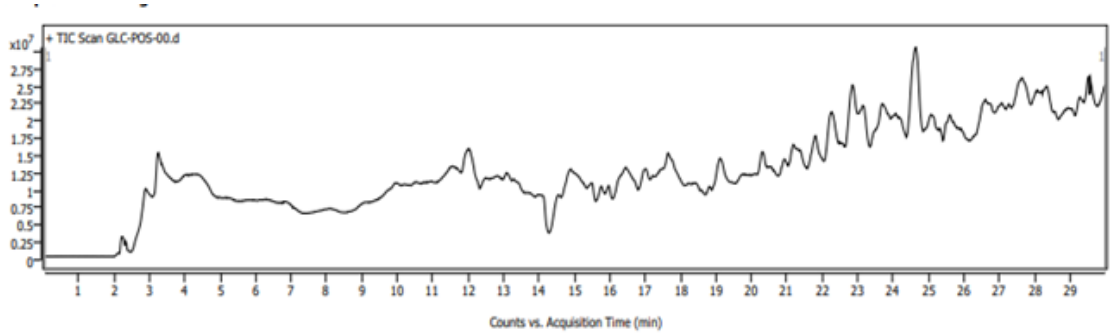

Fig. 2. LC-HRMS of *Gnidia glauca* Leaves Chloroform extract

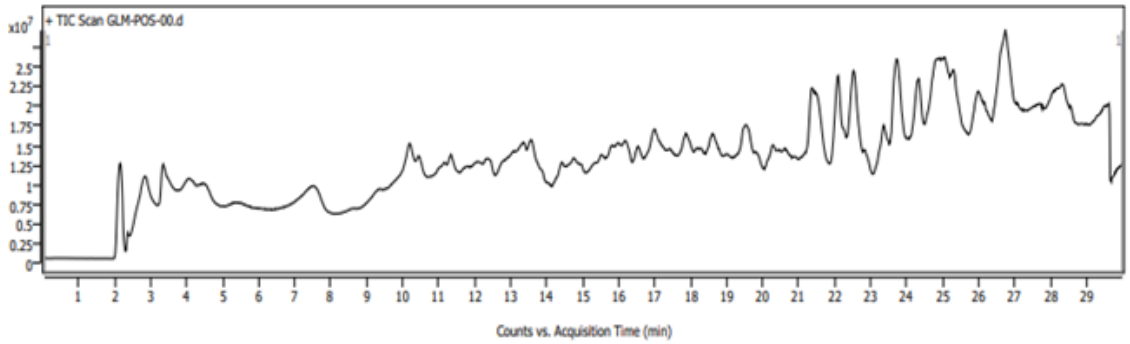

Fig. 3. LC-HRMS of *Gnidia glauca* Leaves Methanol extract

LC- HRMS and Phytochemicals analysis of *Gnidia glauca* L. leaf crude extract with different solvents

Discussion

A plethora of phytochemical screenings of botanical specimens has been conducted, unveiling the presence of myriad clusters of chemical compounds (1). It has also been documented that rotenone, a chemical constituent discovered within *Gnidia glauca*, possesses ichthyotoxic properties and possesses the remarkable ability to induce the excruciating loss of teeth. It appears that various phytochemicals present in *Gnidia glauca* are exerting an influence, as evidenced by numerous scholars. *Gnidia glauca* hampers the activity of amylase and glycosidase, yielding favorable outcomes (6).

Components of phytochemistry

Investigation of phytochemicals was conducted in three solvents of the leaf of *Gnidia glauca* (Table no. 1). When compared to the extracts of *Gnidia glauca* prepared with alcohol, chloroform, or methanol, there exist numerous medical applications for these compounds. The range of secondary metabolites produced by *Gnidia glauca* is delineated below, alongside some of their biological functions. Due to their extensive employment in medicine, *Thymelaeaceae* plants have been the subject of phytochemical studies, and there have also been reports on their toxicity (5).

The phytochemicals in three preparations of *Gnidia glauca* leaves were qualitatively analyzed. The methanolic extract of *Gnidia glauca* exhibited the highest extraction of phytochemicals when compared to chloroform and alcohol extracts. The phytochemical screening of all leaf extracts unveiled the presence of flavonoids, steroids, tannins, and terpenoids. The phytochemical screening reveals that these chemicals possess the potential to be employed for a variety of therapeutic purposes.

Methanol, chloroform, and alcohol were utilized to extract 20 grams of powdered dry leaves, with 200 milliliters of solvents. The leaves of *Gnidia glauca* yielded a total of 1-2 grams of crude extract. One gram of the crude extract was utilized for solvent partition using solvents with varying polarities. In this investigation, solvents with increasing polarity such as chloroform, alcohol, and methanol were employed. The solvent partition was repeated until the solvent in the thimble became clear, indicating the completion of the extraction process. When compared to the other partitions, the methanol partition yielded the highest amount. This finding revealed the presence of polar chemicals in *Gnidia glauca* leaves. LC-HRMS analysis was performed on each partition. LC-HRMS analysis was employed to identify the chemical components found in *Gnidia glauca* leaf extracts. Table 2 enumerates the active principles in solvent extracts of *Gnidia glauca* leaves, alongside their retention time (RT), molecular formula, and molecular mass (MW).

Phytochemical profiling using LC-HRMS

The foliage of *Gnidia glauca* was individually extracted utilizing the Sohxlet extraction technique with three dissimilar polarity solvents (chloroform, methanol, and alcohol). The LC-HRMS data (retention time, molecular formula, and m/z) of the tentatively annotated peaks are documented in Tables 2, 3, and 4 subsequent to the generation of the extracts being subjected to comprehensive LC-HRMS investigations. On-line databases (METLIN, KNApSacK, PubChem, NIST Chemistry WebBook) or earlier literature reporting on the LC-MS analysis of phytochemicals were employed to compare the present study. All of these compounds were discovered in the alcoholic extract of *Gnidia glauca* leaves, namely 3-Phenylpropyl glucosinolate, DL-2-Aminoadipic acid, Prasterone sulfate, N'-Hydroxyneosaxitoxin, Zwittermicin A, Famciclovir, Dimepiperate, Monoglyceride citrate, Pyranodelphinin A, N2,N2-Dimethylguanosine, Decarbamoylneosaxitoxin, (R) Pantothenic acid 4'-O-b-D-glucoside, N-heptanoyl-homoserine lactone, Musca-aurin-VII, Trichotomine, Hydralazine, Erinapyrone C, Kiwiionoside, (+)-Gallocatechin, Ginkgolide, Garcimangosone D, Khellolglucoside, 6-Un-

decanone, Lansiumamide C, Aloesol 7-glucoside, 3-Feruloyl-1,5-quinolactone, Nicotine imine, 16-hydroxy hexadecanoic acid, 3R-hydroxy-octadecanoic acid, 10-Hydroxymyristic acid methyl, Cyclic de-hypoxanthine futalosine. In the methanolic extract, N'-Hydroxyneosaxitoxin, Mycinamicin IV, Lycoperoside D, Aloesol 7-glucoside, Tiamulin, 3-Feruloyl-1,5-quinolactone, 4,4-Difluoropregn-5-ene-3,20-dione, Nicotine imine, 16-hydroxy hexadecanoic acid, Prasterone sulfate, Zwittermicin A, Famciclovir, Decarbamoylneosaxitoxin, Musca-aurin-VII, Hydralazine, Kiwiionoside, (+)-Gallocatechin, Ginkgolide C, Garcimangosone D, Khellol-glucoside, 10,16-dihydroxy-palmitic acid, Cyclic de-hypoxanthine futalosine were present. Meanwhile, in the chloroformic extract, the compounds identified were cortisol 21-sulfate, ACRL Toxin II, Mycinamicin IV, Pithedu-loside A, Tiamulin, Nicotine imine, 16-hydroxy hexadecanoic acid, 3 Irinotecan, Famciclovir, (+)-Gallocatechin, Garcimangosone D, 3R-hydroxy-octadecanoic acid, 3-Hydroxyindo-lin-2-one, 1-(m-Methoxycinnamoyl) pyrrolidine, Cyclic de-hypoxanthine futalosine, Proglumide.

Some of the compounds were present in all three extracts and exhibited similar activity. Recently, *Gnidia glauca* leaves have been documented. The overall phenolic content of the active extracts (126.25 0.20 g GAE/mg) and flavonoid content (25.75 0.10 g CE/mg) were both substantial (25). Figure 1, Figure 2, and Figure 3 illustrate the peak of the compound.

Antioxidant properties

The primary phenolic content of the methanolic extract derived from the leaf *of Gnidia glauca*, which possesses noteworthy antioxidant properties, amounted to 203.3 GAE/g. Its IC50 for ABTS was 16.3 g/mL, while for nitric oxide radical scavenging it was 360.8 g/mL. Furthermore, at the 30-minute mark, a FRAP value of 993.7 m TE/mg was observed, and the total antioxidant activity was evaluated at 142.5 mg AAE/g (27). Similar patterns were observed in the alcoholic extracts of *Gnidia glauca* leaf, which exhibited substantial phenolic and flavonoid content. The alcoholic extracts of *Gnidia glauca* leaves demonstrated second order rate constants of 3.73 106 in the case of pulse radiolysis-generated hydroxyl radical scavenging. The leaf extract in methanol displayed effective DPPH, superoxide, and nitric oxide radical scavenging activities (7).

Pesticidal and larvicidal properties

In Kenya, the utilization of *Gnidia glauca* leaves as an insecticide has been documented (13). The eggs of the teak defoliator *Hyblaea puera* Cramer were significantly eradicated by an aqueous extract derived from the leaves and bark of *Gnidia glauca*, with mortality rates reaching 44.4% and 45.7%, respectively (20). To explore the antileukemic and piscicidal properties of *Gnidia glauca*, dried ground roots were subjected to extraction using 95% ethanol at ambient temperature, while being gently agitated for duration of 24 hours. In order to isolate the piscitoxic fraction, known as gnidiglaucin ($C_{32}H_{46}O_{10}$), the extract was subsequently partitioned using varying proportions of a chloroform-water mixture. However, the isolated compound did not exhibit any inhibitory effects in an in-vivo test for antileukemic activity (P388) (34). Hexane and chloroform extracts obtained from the dried bark of *Gnidia glauca* demonstrated a modest larvicidal effect on mosquito larvae, whereas extracts derived from the fresh bark of the plant exhibited more pronounced larvicidal effects on *Aedes aegypti* second instar larvae. Remarkably, the chloroform extract of fresh bark displayed its maximum activity, resulting in 100% mortality, within a matter of minutes. Bioassay-guided fractionation revealed that the larvicidal activity is primarily attributed to substances such as bicoumarin and Pimelea factor P2 (2). The methanolic extract of *Gnidia glauca* leaves exhibits properties that are beneficial in the management of diabetes (6). Among the leaf extracts of *Vitex negundo*, the 1% chloroform extract demonstrates the highest larval mortality rate (10.30%), surpassing the acetone leaf extract

(7.7%) and the methanol leaf extract (3.09%) (32). Furthermore, it outperforms the artificial diet, which is both costly and time-consuming, in investigating growth factors necessary for the development of early-stage larvae (21).

Antimicrobial properties

Plant pathogenic fungi are the main reason for significant losses in crop output as well as in the farmers' income. Thus, it is crucial to produce low-cost, herbal antifungal medications that are also environmentally friendly. A plant pathogenic fungus called *Phytophthora parasitica*, which causes pineapple heart rot, was variably inhibited by aqueous extracts of different sections of *Gnidia glauca*. The *Gnidia glauca* seeds, leaves, and barks displayed an inhibition up to 19.16, 15.90, and 23.46%, respectively, at a concentration of 5%. Similar to this, increased activity was seen at 10%, which is equivalent to 28.47, 34.59, and 33.60% for seed, leaves, and bark, respectively (20). Recently, a strong anticariogenic activity of the methanolic extract of *Gnidia glauca* leaves against *Streptococcus mutans* was discovered. The total phenolic and flavonoid contents of the active extracts were both high (126.25 0.20 g GAE/ mg and 25.75 0.10 g CE/mg, respectively) (12). When compared to leaf and flower extracts, *Gnidia glauca* bark extract has greater antibacterial action against pathogens that cause urinary tract infections, such as *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus,* and *Enterococcus faecalis* (25).

Nano particles properties

The flower extract of *Gnidia glauca* facilitates the production of gold nanoparticles owing to its abundant phenolic and flavonoid constituents. This process exhibits remarkable expeditiousness, as it can be accomplished entirely within a time frame of fewer than 20 minutes. The ultimate form of the AuNPs consists of diminutive spheres with a median diameter of 10 nm. Through the utilization of high resolution transmission electron microscopy and various other methods of characterization, peculiar structures such as Nano-triangles were also observed (6).

Conclusion

Gnidia glauca, an eminent botanical specimen, possesses remarkable ethno medicinal properties, encompassing anticoagulant, antioxidant, and anthelmintic activities. Furthermore, it serves as a biomarker for oral cancer patients undergoing cancer treatment. This multifaceted plant exhibits allergenic, anesthetic, antibacterial, anticancer, ant mutagenic, antiseptic, antispasmodic, antitumor, and tyrosinase inhibitory qualities. Additionally, it functions as a flavor enhancer, insecticide, nematicide, pesticide, sedative, and termiticide, while also possessing antipruritic and fungicidal attributes. Notably, it demonstrates host-specific pathotoxicity, leading to cellular demise in both plant and animal organisms. Moreover, it showcases antioxidant and hypocholesterolemic properties, alongside its nematicidal, pesticidal, and antiandrogenic effects. It imparts a delightful flavor and exhibits hemolytic and 5-alpha reductase inhibitory activities. Furthermore, it displays commendable insecticidal, herbicidal, and fungicidal properties, as well as mild antifungal activity. Phytochemical investigations have unequivocally established the indispensability of *Gnidia glauca* in the daily lives of humans, animals, and plants, ensuring their well-being and vitality.

Conflict of interest

The authors have no conflicts of interest regarding this investigation**.**

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