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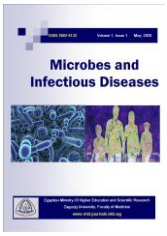

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Original article

Phytochemical profile, antibacterial and antioxidant activities of root extracts of *Gnidia glauca* (Thymelaeaceae)

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ABSTRACT

Background: Plants have been used for many generations for healing purposes, and screening of plant extracts have yielded positive results in the developments of clinically proven drugs. **Objective**: The present study aims to phytochemically investigate and biologically evaluate the antimicrobial potential of *Gnidia glauca gilg* (root used to manage microbial infections in parts of Ethiopia). **Methods**: Root of *Gnidia glauca gilg* was collected from wollega University Garden, Ethiopia. The collected plant material was cleaned to remove the dust particulate and the bark was carefully removed from the root wood. The root back was cut into small pieces and shade dried at room temperature. The dried sample was grounded using electrical miller and sequentially extracted with *n*-hexane and mixture of $CHCl_3$-MeOH (1:1) ratio. Antibacterial activity of the extracts was investigated against *Staphylococcus aureus, Pseudomonas aeruginosa, Shigella dysenteriae, Escherichia coli and Salmonella typhimurium* pathogens. Antioxidant activity of the $CHCl_3$-MeOH (1:1) soluble fraction was evaluated using in vitro 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. In addition, isolation and structural characterizations of the purified compounds were done using chromatographic and spectroscopic techniques. **Results**: The phytochemical constituent identified were flavonoids, alkaloids, tannins, terpenoids, steroids, phenol, saponins and glycosides. Chromatographic separation of $CHCl_3$-MeOH (1:1) soluble fraction afforded two compounds, Tinosporasteryl palmitate (1) and β-amyrin (2). The $CHCl_3$-MeOH (1:1) root extract exhibited promising activities against DPPH, *S. dysenteriae* and *P. aeruginosa*, while a weak inhibition was observed against the rest tested pathogens. **Conclusion**: These pieces of work suggest that the root of *Gnidia glauca gilg* has biologically active secondary metabolites that can be used to treat bacterial infections, and can be recommended to conduct detailed phytochemical investigation and cytotoxic effects of the extract.

Introduction

The genus *Glauca* (Family Thymelaeaceae*)* is a common plant genus comprising about 140-152 species distributed mainly in the tropical and southern Africa, although some species are found in Madagascar, western India and Arabia [1-3]. The species of this genus has attracted attention due to its wider application in traditional medicine system in various parts of the world including Africa [4,5]. The extracts of the species were used to manage several ailments. For example, in Ethiopia and Kenya, the leave, root, and

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stem bark of G. *glauca* is used to treat throat aches, abdominal pains, sores, burns, snake bites, contusions, swellings, back and joint pains, indigestion, rabies, and blisters [6]. The most commonly reported secondary metabolites from the leave of *G. glauca* are Terpioides, coumarins, lignans, benzophenone glycosides and flavonoids [7-8]. In Ethiopia the dried root powder of *G.glauca* is mixed with milk and taken orally for seven days to treat rabies and also the root of the plant is pounded and mixed with "teff" powder, then backed and eaten by human for the treatment of Kidney problem [3]. It is also reported that the root of *G. glauca* is used as pesticide, insecticide and molluscicide [9-10]. Despite the wide usage of this plant in the traditional circles for the treatment of various ailments, the phytochemical information pertaining to this plant and its biological activity has been limited to the aerial parts only. Thus, in our continuing efforts to search phytochemicals with biological activities from medicinal plants of Ethiopia here in this paper we reported the isolation of two compounds from the root extracts of *G.glauca* along with antibacterial and antioxidant activities.

Materials and methods

Plant material

For this laboratory-based study, the fresh root of the *G. galauca* **(Figure 1)** was collected from Wollega University Garden, Ethiopia in October 2021. The plant was identified by botanist and the Voucher specimen (L01/2021) was recorded and kept in the Herbarium at Wollega University Biology Department.

Solvent extraction

The collected root of *G. glauca gilg* was washed with tap water to remove dirty materials. The cleaned root part was dried under shade at room temperature without exposing to direct sun light. The dried plant material was powdered using electrical mill and about 0.5 kg the powdered sample was first soaked in *n*-hexane for 24 hrs. twice to extract the hexane soluble fraction, the solvent was filtered using watt man filter paper (110 mm pore size) followed by cotton plug and solvent was removed under reduced pursuer at 30 ^{O}C with Rota vapor and 4 g of yellowish semisolid substance was obtained, the marc was further extracted exhaustively with $CHCl_3/CH_3OH$ (1:1) for 24 hrs. three times (similar procedure like hexane extract) and 38 g reddish semisolid substance was obtained after removing the solvent under reduced pressure at 45^{O}C. The extracts **(Table 1)** were weighed and stored at 4°C for further analysis.

Four Gram-negative bactria *Escherichia coli (ATCC25922), Pseudomonas aeruginosa* (ATCC27853), *Salmonella typhimurium* (ATCC13311), *Shigella dysenteriae* (ATCC29903) and a Gram-positive *Staphylococcus aureus (ATCC25923),* were used to evaluate antibacterial activities of the extracts. The microorganisms were obtained from Wollega University Department of Biology. Paper disc diffusion method was employed during this study to determine the antibacterial activity of the crude extracts as described by [11]. Briefly a concentration of 50 mg/ml of the plant extracts was prepared from the stock solution and antimicrobial sensitivity testing was done by agar disc diffusion method assay. The bacterial culture was streaked on Mueller Hinton Agar (MHA) medium and incubated overnight at 37 °C. Cultures of each organism (Bacterial suspension of 1.0×10^8 Colony-forming unit (CFU per mL) were inoculated separately on the surface of Mueller Hinton agar plates by surface spreading using a sterile cotton swab and evenly spread over the entire surface of agar plate to obtain uniform inoculums. About 50 µl of the 50 mg/ml of each of the crude extract solution was delivered onto a sterile paper disc (Whatman grade filter paper discs of 6 mm diameter) and 120 µg Erythromycin was used as positive control and Dimethyl sulfoxide (DMSO) was used as negative control. The set up was incubated for 24 h at 37°C. After 24 h incubation, the zones of inhibition were measured using a ruler and the results reported in millimeters (mm). All the tests were run in triplicates and the average result was taken.

Antioxidant activity

The free radical scavenging activity of the $CHCl_3$-MeOH (1:1) root bark extract of *G. glauca* was determined using DPPH radical scavenging method according to [12].

A solution of DPPH in 99% methanol (0.1mM or 0.04g) was prepared in 100 mL volumetric flask and also 1 g of $CHCl_3$-MeOH (1:1) *G.glauca* root bark extract was prepared in 25 mL of 99% methanol to make 40 mg/mL stock solution. Then, 1 mL of DPPH solution was mixed to different concentrations (4 µg/mL, 8µg/mL, 12µg/mL, 16 µg/mL 20µg/mL and 24 µg/mL) of *G.glauca* root bark extract. The reaction mixture was shaken thoroughly and left in the dark at room

temperature for 30 minutes. After 30 minutes the absorbance of the mixture was measured at 517nm that was the maximum wavelength of DPPH and it's compared to the standard antioxidant ((ascorbic acid (vitamin C)) Which was prepared following the same experimental procedure with sample preparation. Percentage inhibition (% RSC) of $CHCl_3$-MeOH (1:1) of *G.glauca* root bark extract in methanol solution was calculated from the absorbance of control and absorbance of the sample according to the following equation:

$$\% RSC = (AC-AS)/AC \times 100$$

where AC is the absorbance of the control and AS is the absorbance of DPPH in the test samples respectively. Antiradical activity is expressed as fifty percent free radical inhibition capacities (IC_{50}). The experiments were performed in triplicate and the average value was calculated.

Isolation and characterization of compounds

About 5.0 g of $CHCl_3$-MeOH soluble friction of the root extract was adsorbed on silica gel and subjected to column chromatography (glass column 3.4 cm diameter, 40 cm length and 200 g capacity) packed with 150 g of silica gel (100-200 mesh size). The column was eluted with ethyl acetate in *n*-hexane by increasing the polarity of ethyl acetate (0%:100%, v: v). Then, followed with the mixture of ethyl acetate and methanol (90:10, 80:20… 0:100, v: v) solvent system. Eighty fractions, each 50 mL were collected. Fractions of similar TLC profiles were combined and regarded into three sub-fractions (F1-20 as GHEL1, F21-53 as GHEL 2 and F54 -80 as GHEL3). Sub-fraction GHEL2 (*n*-hexane: ethyl acetate (60:40)) showed three well resolved spots on TLC after sprayed with 10% H_2SO_4 solution and heated on hot plate. Up on drying the mass of the combined fraction was 0.25 g. The dried sample was first washed repeatedly with *n*-hexane and re-chromatogram over glass column silica gale by eluting increasing gradient of ethyl acetate in *n*-hexane and resulted two compounds 1(8 mg) and 2 (4.3 mg) respectively. The structure of the obtained compounds 1 and 2 **(Figure 2)** were established based on ^1H and ^{13}C-NMR data **(Table 5)** by comparing with reported literature values.

Phytochemical screening

The confirmatory chemical profile of plant extracts was performed to identify the main classes of phytoconstituents (tannins, flavonoids, alkaloids, saponins, anthraquinones, phenols, glycosides, steroids, and terpenoids) present in the extracts following standard protocols [13].

Test for alkaloids

In this experiment, two tests were performed (Mayer's and Wagner's reaction) to confirm the presences of alkaloid in the extract. The plant extract was dissolved in 100 mL of distilled water, filtered, and heated in water bath with 2 mL of the filtrate and three drops of 1% HCl. Then, 1 mL of the heated mixture was combined with 3 mL of the Mayer-Wagner reagent. The appearance of a cream or brown-red colored precipitate indicated the presence of alkaloids, while in the cases of 2mL of the Wagner reagent was added on 1 mL of the crude extract and the formation of reddish-brown precipitation indicated the presence of alkaloids.

Test for flavonoids and glycosides

About 0.05 g of the plant extract was mixed with 10 mL of ethanol and filtrated. Two mL of the filtrate, concentrated HCl (1 mL), mixed with few strips magnesium ribbon. The formation of a pink or red color indicates the presence of flavonoids. Adding 1 mL of distilled water and NaOH to 0.5 mL of crude extract, the formation of a yellowish color indicated the presence of glycosides.

Test for steroids

About 5mL of the crude extract was mixed with 10 mL of chloroform and 3mL of concentrated sulfuric acid, and the formation of the bilayer (red top layer and greenish bottom layer) reveals the presence of steroids.

Test for terpenoids

The presence of terpenoids was determined by the formation of a reddish-brown color in the test for terpenoids, which included mixing of 0.5 mL of crude extract with 2 mL of chloroform and 3 mL of sulfuric acid.

Test for phenols

About 1 mL of the extract was combined with three drops of FeCl3, and 1 mL of $K_2Fe(CN_6)$. The formation of greenish blue forms confirmed the presence of phenol.

Test for anthraquinone

Borntrager's test: About 0.5 g of the root extract was dissolved in 50 mL of benzene and heated for 10 minutes and then filtered, 10% NH_4OH solution was added and shaken vigorously to confirm the formation of pink, violet, or red color

in ammonia layer that indicate the presences of anthraquinone.

Test for saponins

About 10 mL of the extract and 5 mL of distilled water were combined and agitated. Then, the formation of stable foam for 30 minutes confirmed the presence of saponins.

Test for tannins

About 0.2 g of the plant extract was boiled with 20 mL of distilled water; and 0.1% Ferric chloride was added to the mixture; which was then observed for blue-black coloration indicating the presence of tannins.

Results

The air-dried root bark of *G.glauca* was exhaustively extracted with *n*-hexane followed by mixture $CHCl_3$-MeOH(1:1) and the mass and percentage yield of the extracts indicated in **Table 1**. The percentage yield of the crude extracts was calculated and presented in **(Table 1)**. The extracts were future subjected to phytochemical screening, column chromatography separation, antibacterial and antioxidant activity evaluation presented in **(Tables 2, 3, 4 and 5)** respectively.

phytochemical screening

The Hexane and $CHCl_3$-MeOH root extracts of *G. glauca* was phytochemically screened. The $CHCl_3$-MeOH extract was presented with notable positive phytochemical classes **(Table 2)**, which were evidenced with confirmatory color changes. Flavonoids, alkaloids, terpenoids, steroids and phenols were among classes of compounds detected in the $CHCl_3$-MeOH fraction, while terpenoids and steroids were detected in *n*-hexane extracts **(Table 2)**. Anthraquinone was not found in both cases.

Antibacterial activities

The antimicrobial activities obtained from the extracts of the root of *G,glauca* against the five pathogenic bacterial strains were investigated by the disc diffusion method. As shown in **Table 3**, $CHCl_3$-MeOH soluble fraction exhibited moderate zone of inhiation against the Gram-negative bacterial strains *S. dysenteriae* (16 mm) and *P. aeruginosa* (15mm), while it exerted less significant effects on the Gram-positive strains. On the other hand, the *n*-hexane extract has shown activities to ward none of the bacterial strains. The bacterial strain *S. dysenteriae and P. aeruginosa* with MIC value of 2.5 µg/mL were the sensitive test micro-organisms to the root extract. The experimental data was expressed as Mean ± Standard Deviation (SD). The statistical analysis was carried out using SPSS one-way ANOVA.

Antioxidant activity

In this study, (DPPH) spectrophotometric assay was performed to evaluate the antiradical activity of $CHCl_3$-MeOH extract of *G. glauca*. DPPH is a stable free radical with a strong absorption band in an alcoholic solution at around 517 nm. In the presence of a compound that is capable of either transferring an electron or donating hydrogen, the color of the DPPH solution changes from strong purple to light yellow and hence the absorption of the solution at 517 nm diminutions [14]. Antiradical curves were plotted by putting the samples concentration on x-axis and the relative scavenging capacity on the y-axis and the % RSC value was calculated **(section 2.4)**. The concentration of sample in µg/mL at which the absorbance at 517 nm decreased to half of its initial value is used as the IC_{50} value of the extract. Free radical scavenging activity of the $CHCl_3$-MeOH fraction presented in **Table 4**. Values are means of three replicate determination (n = 3) ± Standard deviation.

Spectroscopic data and structural elucidation of compound 1 and 2

The $CHCl_3$-MeOH roots extract of *G.galuca* was subjected to chromatographic separation on glass column silica gel. On repeated chromatographic separation the extract afforded compounds 1 and 2. Based ^{1}H and ^{13}C-NMR spectrum data compounds 1, 2 identified the structures presented in **Figure 2** as tinosporasteryl palmitate (1) and β-amyrin (2) **Based on the 1H and 13C-NMR spectral data, compounds 1 and 2 have been identified as tinosporasteryl palmitate (1) and β-amyrin (2), respectively, as shown in Figure 2.**, by comparison with previously reported physical and spectral data [15-17]. However, to our knowledge, compound 1 is the first report from *G.glauca*. The ^{1}H and ^{13}C-NMR spectra data of the two compounds presented ^{13}C-NMR in **Table 5**. Tinosporasteryl palmitate: Compound (1) was isolated as white amorphous powder and β-amyrin: Compound (2) isolated as white powder.

Table 1. The percent yield and mass of root extracts of *G.glauca gilg*

G.glauca gilg extract	Mass of extract (g)	Percentage yield (%)
n-Hexane	4	0.8
$CHCl_3$-MeOH (1:1)	38	7.7

Table 2. Phytochemical screening results of n-hexane and $CHCl_3$-MeOH root extracts of *G.glauca*

Class secondary metabolites	Regent used	$CHCl_3$-MeOH extract results	*n*-hexane extract results
Alkaloids	Wagner's reagent	+	-
	Mayers reagent	+	-
Flavonoids	Shinoda	++	-
	Alkaline reagent	++	-
	Lead acetate test	+	-
Phenols	FeCl3	++	
Anthraquinones	Borntrager's	-	-
Glycosides	Keller-kiliani test	+	-
Terpenoids	Salkowski test	++	++
Saponins	Foam test	+	-
Tannins	Ferric chloride test	+	-
Steroids	Liebermann-Burchard's test	+	++

Note: + = mildly positive, ++ = strongly positive, - negative

Table 3. The diameter of zone of inhibition(mm) and MIC value of (µg/mL) of root bark $CHCl_3$-MeOH extract of *G.glauca, E,Coli, S.aureus, S.dysenteria,, P. aeruginosa and S. typhimurium.*

Bacteria strains	Diameter of inhibition zone (mm)		Erythromycin	MIC (µg/ mL)		
	$CHCl_3$-MeOH extract	*n*-hexane extract		$CHCl_3$-MeOH extract	*n*-hexane extract	Erythromycin
E. coli	11±0.2	-	27	-	-	1.2
S. aureus	12±0.6	-	26	-	-	1.2
S. dysenteriae	16±0.3	-	30	2.5	-	1.2
P. aeruginosa	15±0.5		25	2.5	-	1.2
S. typhimurium.	11±0.6	-	30	-	-	1.2

Values are expressed as the mean ± SD (n = 3 or three replicates).

Table 4. Free radical scavenging activities of $CHCl_3$-MeOH root extract of *G.g*lauca.

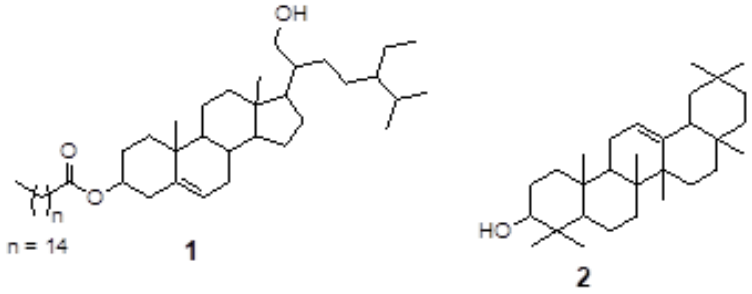
S.No	Concentration of extract and ascorbic acid (µ g/mL)	%Inhibition	
		CHC_3-MeOH	Ascorbic acid (standard
1	4	36.882±0.092	57.10±0.060
2	8	68.981±0.140	77.40±0.110
3	12	83.545±0.120	88.01±0.040
4	16	85.409±0.390	92.16±0.026
5	20	88.326±0.110	98.23±0.030
6	24	92.437±0.020	98.41±0.210

Table 5. 13C NMR spectral data for compounds 1 and 2.

C-1	^{13}CNMR spectral data of 1 (this work)	^{13}CNMR literature data of 2 (100 MHz DMSO-d_6) [16]	C-2	^{13}CNMR spectral data of 2 (this work)	^{13}CNMR literature data of 2 (100 MHz DMSO-d_6) [15]
1	37.24	37.2	1	38.90	38.84
2	33.09	32.8	2	26.89	27.25
3	71.80	71.7	3	78.93	79.30
4	42.31	42.2	4	38.72	38.77
5	140.73	140.7	5	55.48	55.26
6	121.72	121.6	6	18.54	18.32
7	31.99	31.9	7	37.90	38.64
8	31.62	31.6	8	39.44	39.51
9	50.11	50.1	9	48.33	47.92
10	36.15	36.4	10	37.19	36.72
11	21.08	21.0	11	23.64	23.61
12	39.76	39.7	12	122.49	121.70
13	42.01	40.5	13	144.08	145.20
14	56.75	56.7	14	41.92	42.038
15	24.89	24.8	15	28.74	29. 35
16	28.26	28.2	16	23.59	23.16
17	56.04	56.0	17	30.85	29.69
18	11.86	11.9	18	47.84	47.58
19	19.40	19.3	19	39.00	39.08
20	35.70	36.4	20	30.85	30.63
21	65.03	63.0	21	33.19	33.00
22	33.93	33.9	22	38.12	37.02
23	26.0	25.7	23	15.72	15.56
24	45.81	45.8	24	15.72	15.56
25	29.12	29.1	25	15.41	15.47
26	19.83	19.8	26	16.96	16.98
27	19.03	19.0	27	23.64	24.02
28	24.30	24.2	28	16.96	17.09
29	11.90	11.8	29	27.86	28. 14
1’	173.95	167.8	30	27.86	28. 14
2’	36.15	36.1			
3’	33.9	33.7			
4’	29. 63	29.6			
5’	29. 63	29.6			
6’	29. 63	29.6			
7’	29.72	29.8			
8’	29. 63	29.6			
9’	29. 63	29.6			
10’	29.72	29.8			
11’	29.38	29.4			
12’	29.38	29.4			
13’	29.28	29.3			
14’	29.21	27.1			
15’	22.8	22.6			
16’	14.14	14.1			

Figure 1. Photo of the areal part of *G.glauca* on the field (right; the dried root bark of *G,glauca)*

Figure 2. Structures of the compounds isolated from root bark of *G.glauca*



Discussion

The root bark of *G. glauca* was sequentially extracted with *n*-hexane and $CHCl_3$/MeOH (1:1) yields 4 g (0.8%) and 38 g (7.7 %) yellowish and red semisolid substances respectively. The *n*-hexane and $CHCl_3$/MeOH extracts were subjected to phytochemical screening and antibacterial activities, while the $CHCl_3$/MeOH extract was further subjected to column chromatography for further purification and radical-scavenging activity evaluation. Chromatographic separations of $CHCl_3$/MeOH root extract of *G. glauca* resulted two compounds (1 and 2) **Figure 2**. The structure of these compounds was identified based on their spectroscopic data and comparison with reported literature values **(Table 5)**.

The investigated plant was found to contain different phytochemical classes of compounds including flavonoids, alkaloids, phenols, glycosides, terpenoids and steroids; which was confirmed by color change when treated with standard qualitative tests. Among screened classes of compounds flavonoids, alkaloid and phenols were the phytochemicals with significant visible color changes. The $CHCl_3$-MeOH plant extract demonstrated activity against selected microbial strains, with the lowest in-vitro inhibitory concentration (2.5 µg/mL) towards the Gram-negative strains. The sensitivity of the Gram-negative bacteria towards the extract suggests that the mechanism of action of the extract might not be on the cell wall of the Bactria. The activity of the extract may be due to the synergetic effects of the phytochemical constituents detected in the $CHClCl_3$-MeOH fraction, and literature reveals that phytochemical components such as terpenoids, alkaloids, flavonoids, and phenols were the crucial secondary metabolites responsible for efficient anti-microbial capabilities [19-21]. On the other and the antioxidant activities displayed by the $CHCl_3$-MeOH extract at concentration of 24 µg/mL with percenter of inhibition (92.437±0.020) was significant compared to ascorbic acid, indicating the potential of the root bark of this species as natural antioxidants. therefore, the biological activities displayed by the extract from the root bark of *G. glauca* corroborate the traditional use of this plant against various ailments caused by bacteria.

Conclusion

This work aimed at analyzing the phytochemical profile in the root extracts of the plant *G.glauca*, and examine its antibacterial and antiradical effects. The root extract of *G.glauca* plant was found to possess different classes of secondary metabolites evidenced by phytochemical screening results and the isolation of terpen and steroid with observed activity against DPPH free radicals and to some selected bacterial strain specifically Gram-negative microorganisms was an evidence. Thus, the *G.glauca* may be suitable for use in traditional medicine to treat Bacteria and oxidative stress that validating the Ethiopian

folkloric uses of this plant. However, comprehensive evaluations including in vivo antibacterial activity tests are recommended for conclusive decision on potential candidacy of the plant for formulation and medicinal uses.

Declaration of competing interest

All authors declare there is no conflict of interest.

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References

1- Franke K, Porzel A, Schmidt J. Flavone-coumarin hybrids from Gnidia socotrana. Phytochemistry 2002; 61(7): 873-878. https://doi.org/10.1016/S0031-9422(02)00358-8.

2- Ghosh S, Ahire M, Patil S, Jabgunde A, Bhat Dusane M., *et al* Antidiabetic activity of Gnidia glauca and Dioscorea bulbifera: potent amylase and glucosidase inhibitors. Evid Based Complementary Altern Med 2011; 2012: 1010. |https://doi.org/10.1155/2012/929051.

3- Kalbessa A, Dekebo A, Tesso H, Abdo T, Abdissa N, Melaku Y. Chemical constituents of root barks of Gnidia involucrata and evaluation for antibacterial and antioxidant activities. J. Trop. Med 2019.

4- Amarajeewa BWRC, Mudalige AP, Kumar V. Chemistry and mosquito larvicidal activity of Gnidia glauca. Proceedings of the Peradeniya University Research Sessions, Sri Lanka 2017; 12(1): 101-102. doi: 10.1155/2012/929051.

5- Avana-Tientcheu ML, Solefack MCM, Kamga YB, Nguetsop VF. Vulnerability assessment of Gnidia glauca (Thymelaeaceae) exploitation, traditional uses and domestication potential in the community forest of Kilum-Ijim, North Western Cameroon. JENE 2018; 10(8): 182-191. https://doi.org/10.5897/JENE2018.0703.

6- Amenu E. Use and Management of Medicinal plants by Indigenous People of Ejaji Area (Chelya Woreda) West Shoa, Ethiopia: An Ethnobotanical Approach (Thesis submitted for the degree of master in science in dryland biodiversity, Addis Ababa University), Ethiopia. Applied Pharmaceutical Science 2007.

7- Ferrari J, Terreaux C, Sahpaz S, Msonthi JD, Wolfender JL, Hostettmann K. Benzophenone glycosides from Gnidia involucrata. Phytochemistry 2000; 54(8): 883-889. https://doi.org/10.1016/S0031-9422(00)00046-7.

8- Kharat SS, Kumkar PB, Rajpure SR, Sonawane KS. Qualitative phytochemical screening of Gnidia glauca (Fresen) Gilg. plant extract. IJPBS 2023; 4(4): 144-148.

9- Rao SB, Jayanthi M, Yogeetha R, Ramakrishnaiah H, Nataraj J. Free radical scavenging activity and reducing power of Gnidia glauca (Fresen.) Gilg. J. Appl. Pharm. Sci 2013; 3(6): 203-207. DOI: 10.7324/JAPS.2013.3634.

10- Ghosh S, Parihar VS, Dhavale DD, Chopade B A. Commentary on therapeutic potential of Gnidia glauca: A novel medicinal plant. Med chem 2015; 5(8): 351-353. http://dx.doi.org/10.4172/2161-0444.1000285.

11- Alwahibi MS, & Perveen K. Chemical analysis by GC-MS and in vitro antibacterial activity of Alkanna tinctoria extracts against skin infection causing bacteria. Biomed. Res 2017; 28(18): 7946-

7949. ISSN 0970-938X www.biomedres.info.

12- Pellati F, Benvenuti S, Magro L, Melegari M, Soragni F. Analysis of phenolic compounds and radical scavenging activity of Echinacea spp. JPBA 2004; 35(2): 289-301. https://doi.org/10.1016/S0731-7085(03)00645-9.

13- Parbuntari H, Prestica Y, Gunawan R, Nurman MN, Adella F. Preliminary phytochemical screening (qualitative analysis) of cacao leaves (Theobroma cacao L.). EKSAKTA: Berkala Ilmiah Bidang MIPA 2018; 19(2): 40-45. https://doi.org/10.24036/eksakta/vol19-iss2/142.

14- Yang J, Guo J, Yuan J. In vitro antioxidant properties of rutin. LWT-JFST 2008; 41(6): 1060-1066. https://doi.org/10.1016/j.lwt.2007.06.010.

15- Ogwuche CE, Amupitan JO, Ndukwe IG, Ayo RG. Isolation and biological activity of the triterpene β-amyrin from the aerial plant parts of Maesobotrya barteri (Baill). Med Chem 2014; 4: 729-733. DOI: 10.4172/2161-0444.100022.

16- Singh V, Ali M, Pahuja C. A new steroidal ester from Tinospora cordifolia Miers. stems. Der Pharmacia Lettre 2016; 8(20): 47-51. http://scholarsresearchlibrary.com/archive.html.

17- Sannabommaji T, Kumar V, Poornima DV, Gajula H, Rajashekar J, Manjunatha T, *et al*. Phytochemical Analysis with Special Reference to Leaf Saponins in Gnidia glauca (Fresen.) Gilg. Biotechnological Approaches for Medicinal and Aromatic Plants: Conservation, Genetic Improvement and Utilization 2018; (27)1-287. https://doi.org/10.1007/978-981-13-0535-1_12.

18- Odeleye OM, & Oyedeji AO. Antibacterial activity of crude and fractions of Momordica foetida leaf extracts. Int. J. Pharm 2008; 2(2): 75-78.

19- Mayeku PW, Hassanali A, Kiremire BT, Odalo JO, Hertweck C. Anti-bacterial activities and phytochemical screening of extracts of different parts of Thalictrum rhynchocarpum. Afri J Trad Complement Altern Med 2013; 10(5): 341–344. doi:10.4314/ajtcam. v10i5.20.

20- Mabhiza D, Chitemerere T, Mukanganyama S. Antibacterial properties of alkaloid extracts from Callistemon citrinus and Vernonia adoensis against Staphylococcus aureus and Pseudomonas aeruginosa. Int J Med Chem 2016; 1–7. http://dx.doi.org/10.1155/2016/6304163.

21- Farhadi F, Khameneh B, Iranshahi M, Iranshahy M. Antibacterial activity of flavonoids and their structure–activity relationship: an update review. Phytother Res 2010; 33(1): 13–40. doi:10.1002/ptr.6208.