Antibacterial screening and GC-MS profile of leave and stem fractions of *Calotropis procera* (Linn)

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Abstract

Traditional medicine also known as Indigenous or folk medicine comprises of knowledge systems that developed over generations within various societies before the era of modern medicine. The column chromatography was used to collect the crude fractions; solvents like petroleum ether, chloroform and methanol were used. The antibacterial activities of the crude fractions *of Calotropis procera* (leaf and stem) were evaluated in this study using some selected microorganisms like *Escherichia coli, Shigella dysenteriae* ATCC 24162, Salmonella typhi and Klebsiella pneumoniae ATCC 34089, whereby the plant was extracted using (acetone, methanol and aqueous). The paper disc method was used after which the zone of inhibition around the discs was estimated. The results showed that in the leaf of the plant; *E. coli* with aqueous-methanol fraction and *K. pneumoniae* ATCC 34089 with acetone-petroleum ether fraction had the highest yield of 30mm respectively while in stem; *K. pneumoniae* ATCC 34089 with methanol-methanol fraction had the highest yield of 25mm. The structural elucidation of the bioactive compounds in the extracts were evaluated using GC-MS which reveals the chemical compounds like phenol, methyl palmitate, phthalic acid, 9-octadecenoic acid and other compounds known for valuable antimicrobial, biological activities and antioxidant properties. The leaves and the stem are having good chemical compounds that can be responsible for the antimicrobial property observed.

Keywords: analysis leaves; *Calotropis procera;* crude fractions; gas chromatography; mass spectrometry; phytochemistry stem.

Evaluación antibacteriana y perfil cromatográfico (CG-EM) de fracciones de hojas y tallo de *Calotropis procera* (Linn)

Resumen

La medicina tradicional, también conocida como medicina ancestral o popular, comprende sistemas de conocimientos que se desarrollaron a lo largo de generaciones dentro de varias sociedades antes de la era de la medicina moderna. La cromatografía en columna fue utilizada para recolectar fracciones crudas; se utilizaron disolventes como éter de petróleo, cloroformo y metanol. Las actividades antibacterianas de las fracciones crudas de Calotropis procera (hojas y tallo) se evaluaron en este estudio, utilizando algunos microorganismos seleccionados como *Escherichia coli, Shigella dysenteriae* ATCC 24162, *Salmonella typhi y Klebsiella pneumoniae* ATCC 34089; para tal fin, la planta fue extraída usando acetona y metanol acuoso. El método de difusión en disco de papel fue usado después de que la zona de inhibición alrededor de los discos fue estimada. Los resultados mostraron que en las hojas de la planta, *E. coli* con la fracción acuoso-metanol y *K. pneumoniae* ATCC 34089 con la fracción del éter de petróleo-acetona, tuvieron el rendimiento más alto de 30mm respectivamente; mientras que en el tallo, K. pneumoniae ATCC 34089 con la fracción del metanol-metanol tuvo el rendimiento más alto de 25mm. La elucidación estructural de los compuestos bioactivos en los extractos fue evaluada usando CG-EM, identificándose compuestos químicos como fenol, palmitato metílico, ácido ftálico, ácido 9-octadecenoico y otros compuestos conocidos como antimicrobianos valiosos, con actividades biológicas y propiedades antioxidantes. Las hojas y el tallo han presentado buenos compuestos químicos, que pueden ser responsables del efecto antimicrobiano observado.

Palabras Clave: análisis de hojas; Calotropis procera; fracciones brutas; cromatografía de gases; espectrometría de masas; fitoquímica

INTRODUCTION

The World Health Organization (WHO) defines traditional medicine as the sum of total of the knowledge, skills, and practices based on the theories, beliefs and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health as well as in the prevention, diagnosis, improvement or treatment of physical and mental illness (1).

The use of herbal medicine as alternative therapy has prevalent throughout the world due to the growing resistance of pathogens to conventional antibiotics (2). The need for more potent, safe and affordable drugs has led to intensified research into herbal drugs, the result of which is the introduction of new herbal preparation for therapeutic uses (3).Medicinal plants are frequently used as remedies for many infectious diseases (4). The treatment and control of diseases by the use of the available medicinal plants in a locality have been helpful and of a priority to majority of urban and rural dwellers in healing various diseases because of the reliability and stability in plant products for healing (5).

Calotropis procera (Apple of Sodom) is a shrub or small tree, which has become a serious weed in pastures and overgrazed rangelands. It is a native to West Africa as far as south as Angola, North and East African, Madagascar, the Arabian Peninsula, Southern Asia, India and China to Malaysia. Calotropis was formerly placed in the family of Asclepiadaceous (the milkweed family), which is now considered a subfamily of the Apocynaceae (6).

MATERIALS AND METHOD

Collection of plant samples

Apparently healthy plant namely Calatropis procera were collected from Ado-Ekiti, Ekiti State Nigeria.

The plants parts leave and stem were air-dried for 5 weeks at room temperature (25 + 2 oC) and then ground to powder with a mechanical grinder (Thomas Wiley machine, model 5 USA). Powders (200gs) of each plant were extracted with 1litre of sterile aqueous water, ethanol, methanol and acetone separately at room temperature (25 + 2 oC). They were labeled as crude extracts.

Antibacterial Screening of the Crude Fractions

The evaluation of antimicrobial activity was performed for all fractions by the paper disc agar diffusion method following the standard rules of antimicrobial sensitivity tests by the Clinical and Laboratory Standards Institute (7). The antibacterial effect of the fractions was carried out whereby the discs were previously impregnated with the plant crude fractions and placed on the sterile prepared medium. The plates were incubated at 37 oC for 24 - 48 hours. The sensitivity of the test organisms to each of the extracts was indicated by clearing around each disc. The diameter as an index of the degree of sensitivity was measured with a transparent plastic ruler.

Column chromatography and fraction extracts

Glass wool was placed at the outlet of a column after which, one gram of plant extract powder was weighed into it and subjected to column chromatography (30 x 8 cm column) using 60 g of silica gel 60 F254 (Merck, 0.020 mm thickness). The column was successively eluted first with petroleum ether (150 ml) and then with chloroform (80 ml): methanol (2 ml) (40:1) and finally with 100% methanol (150 ml). Each 100 ml eluent was collected into a round bottom flask (250 ml) capacity and distilled to obtain fractions. The fractions collected were numbered. GC-MS (Gas Chromatography-Mass Spectrophotometry) analysis was carried out in GCMS-QP2010 PLUS Shamadzu.

Antibacterial screening of crude fractions

The antibacterial screening of the crude fractions of Calotropis procera leaf was examined; some chemicals were used in the fractionalization of the crude sample, which are methanol, chloroform and petroleum ether. Methanol, aqueous and acetone leaf fractions showed the highest zone of inhibition were: 30, 20 and 20 mm respectively. The use of petroleum ether do not show any significant antibacterial effects against the isolates while aqueous crude fractions shows an effective antibacterial activity against S. aureus at zone of inhibition of 15 mm (Table 1).

Table 1: Antibacterial screening of crude fractions of Calotropis procera leaf (mm)

ORGS	ACM	ACC	ACP	MM	MC	MP	AQM	AQP	AQC	EC	EP	EM
E.coli	-	-	-	4	20	2	30	-	20	-	-	-
S. aureus	-	-	-	10	5	-	-	-	15	-	-	-
E.coli												
ATCC 35218	-	-	-	-	20	-	-	-	-	-	-	-
S. typhi	20	15	20	18	5	-	-	-	-	10	5	7
S. dysenteriae	20	-	-	14	-	-	-	-	-	4	14	-
S. dysenteriae												
ATCC 24162	2	-	-	22	14	2	-	-	-	-	-	-
K. pneumonia												
ATCC 34089	20	-	30	-	-	-	-	-	-	-	-	-
K.pneumoniae	24	2	-	-	-	-	-	-	-	-	-	-

ACM- Acetone methanol, ACC- Acetone Chloroform, ACP-Acetone Pet. Ether, MM-methanol methanol, MC-Methanol Chloroform, MP-Methanol pet. Ether, AQC-Aqueous Chloroform, AQP Aqueous Pet.ether, AQM- Aqueous Methanol, EM-Ethanol Methanol, EC- Ethanol Chloroform, EP- Ethanol Pet. Ether. (-): did not show inhibition zone.

Table 2 shows the antibacterial screening of the stem crude fractions to indicate the effectiveness of various bioactive or metabolite fractions found in the crude stem extracts of the plant (C. procera). During this research, also methanol was found to show a significant antibacterial effect on K. pneumoniae at zone of inhibition of 25 mm. Both methanol and aqueous crude fractions have shown good antibacterial activities.

Table 2. Screening of antibacterial crude fractions of Calotropis procera stem (mm)

ORGS	ACM	ACC	ACP	MM	MC	MP	AQC	AQP	AQM	EM	EC	EP
E. coli	-	-	-	-	-	-	-	-	5	-	-	-
S. dysenteriae ATCC 24162	-	-	-	-	-	-	10	3	3	6	-	-
K. pneumonia ATCC 34089	-	-	-	25	-	9	-	-	-	-	-	-
S. typhi	-	5	10	2	-	7	-	-	21	6	-	-

ACM- Acetone methanol, ACC- Acetone Chloroform, ACP-Acetone Pet. Ether, MM-methanol methanol, MC-Methanol Chloroform, MP-Methanol pet. Ether, AQC-Aqueous Chloroform, AQP-Aqueous Pet.ether, AQM- Aqueous Methanol, EM-Ethanol Methanol, EC- Ethanol Chloroform, EP- Ethanol Pet. Ether. (-): did not show inhibition zone.

Tables 3-8 and Figures 1 -2, indicate the results of GC-MS analysis with peaks of secondary metabolites of the crude fractions. Those shows diverse peaks which implies different chemical compounds that are been found in the fractions of C. procera.

The chemical compounds of the crude fractions were determined and listed out with their retention time and the concentration of the chemical compounds in percentage. The secondary metabolites identified were phenol, methyl palmitate, 9-octadecenoic acid, phthalic acid, dimethyl sulfoxide, phytol and among others.

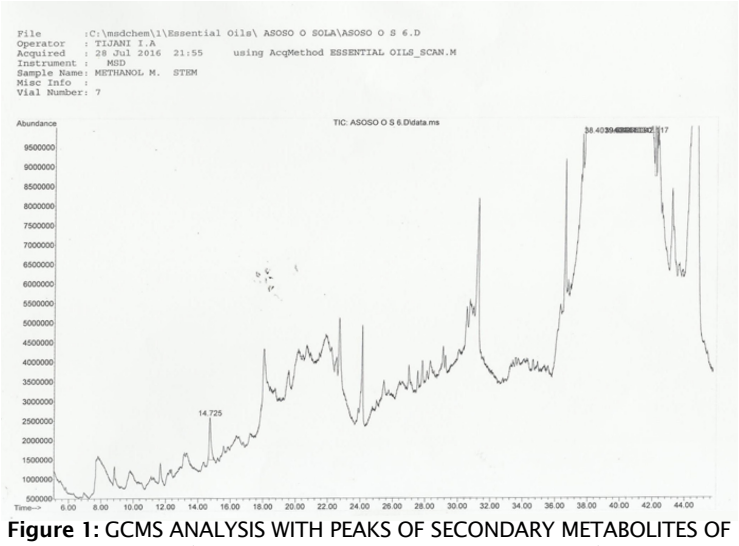

Figure 1: GCMS ANALYSIS WITH PEAKS OF SECONDARY METABOLITES OF MMS (METHANOL METHANOL STEM FRACTION)

Table 3. Chemical compounds of methanol- methanol stem fraction of C. procera, identified by GC-MS analysis.

S/N	Chemical compounds	Retention Time (mins)	Concentration (%)
1	phenol	14.725	1.761
2	Methyl	38.401	7.053
3	9.12 octadecadienoic acid (z,z)- Methyl ester	39.625	2.814
4	9- octadecadienoic acid	39.666	4.602
5	octadecadienoic acid	40.184	34.65
6	Methyl 19,22- hepatadeca dienoate	40.225	8.98
7	9 – octadecadienoic acid	40.7033	31.16
8	Tetradecanoic acid	41.080	4.21
9	Phthalic acid	42.117	4.76

Identified by GC-MS analysis

Table 4. Chemical compounds of methanol- aqueous stem fraction of C. procera, identified by GC-MS analysis

S/N	CHEMICAL COMPOUNDS	RETENTION TIME (min)	CONCENTRATION(%)
1	Hydrazine	7.797	0.92
2	Thietane	7.876	0.86
3	Dimethyl Sulfoxide	9.282	13.23
4	2(5H)-Furanone	11.905	13.53
5	Triacetin	23.798	1.22
6	4-methyl-5-methoxy-1,2,4-triazole-3-thione	24.018	1.33
7	Methyl palmitate	38.386	4.40
8	9-octadecenoic acid	39.650	0.69
9	Methyl palmitate	39.807	2.08
10	Benzenemethanamine	41.512	11.17
11	Phthalic acid	42.109	18.97
12	1H-Indole	42.219	22.26
13	2-methyl-7-phenylindole	42.345	7.24
14	(2,3-Diphenylcyclopropyl) methylphenyl sulfoxide	43.060	2.09

Table 5. Chemical compounds of chloroform-aqueous leaf fraction of C. procera, identified by GC-MS analysis

S/N	CHEMICAL COMPOUNDS	RETENTION TIME(min)	CONCENTRATION (%)
1	Dodecane	15.802	1.67
2	Phenol	29.061	2.79
3	Cyclohexadecane	31.151	1.55
4	9-Octadecene	36.744	1.68
5	Dodecane	36.744	1.22
6	Phthalic acid	37.435	4.64
7	Phthalic acid	37.749	37.68
8	Methyl palmitate	38.401	12.72
9	9-octadecenoic acid	39.658	12.72
10	Methyl palmitate	39.823	8.60
11	Phthalic acid	42.109	6.94

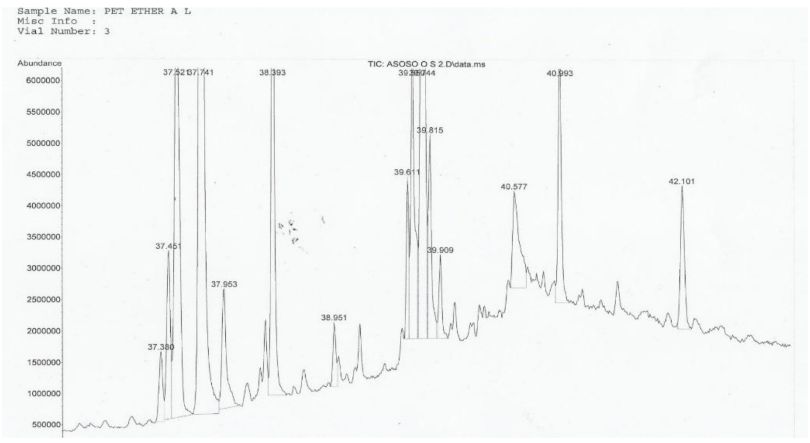

Figure 2: GCMS analysis with peaks of secondary metabolites of pal (pet-ether acetone leaf fraction)

Table 6. Chemical compounds of pet-ether acetone leaf fraction of C. procera, identified by GC-MS analysis

S/N	CHEMICAL COMPOUNDS	RETENTION TIME (min)	CONCENT RATION (%)
1	2-Hexadecene	37.380	1.29
2	11-tetradecyn-1-ol acetate	37.451	2.94
3	2-pentadecanone	37.521	10.40
4	Phthalic acid	37.741	24.54
5	3,7,11,15-tetramethyl-2-hexadecenol-ol	37.953	2.70
6	Methyl palmitate	38.393	7.66
7	Hexadecanoic acid	38.951	0.97
8	9,12-octadecadienoic acid(z,z)-methyl ester	39.611	1.80
9	7,10,13-hexadecatrienoic acid	39.650	5.53
10	Phytol	39.744	28.64
11	Methyl palmitate	39.815	2.75
12	Cyclopentanone	39.909	1.11
13	Bromacetic acid	40.577	3.88
14	4,8,12,16 tetramethylheptadecan-4-olide	40.993	3.88
15	Phthalic acid	42.101	2.66

The table 6 shows the explanation of the Figure 1. above, GCMS analysis with peaks of secondary metabolites of pal (pet-ether acetone leaf fraction)

Table 7. Chemical compounds of methanol-methanol leaf fraction of C. procera, identified by GC-MS analysis.

S/N	CHEMICAL COMPOUNDS	RETENTION TIME(min)	CONCENTRATION(%)
1	Dimethyl sulfoxide	9.400	91.44
2	3-acetoxy-3-hydroxypropionic acid	16.878	5.05
3	Methyl palmitate	38.401	3.51

Table 8. Chemical compounds of methanol- aqueous leaf fraction of C. procera, identified by GC-MS analysis.

S/N	CHEMICAL COMPOUNDS	RETENTION TIME (min)	CONCENTRATION(%)
1	Butane	9.965	65.81
2	n- hexadecanoic acid	39.116	26.20
3	9-octadecenoic acid	40.074	2.20
4	Octadecanoic acid	40.208	5.79

DISCUSSION

Antibacterial activities of the crude fractions showed that this plant can be used for curing many diseases such as pneumonia, dysentery among others. The results demonstrated that the crude extracts were more efficient than the fractions, this findings might suggest that Phytochemical constituents in combination may be having synergy in their efficacy, which is in agreement with many other report that have shown higher antibacterial potency of crude extracts as compared to the fraction (8).

The difference of various plant extracts in the antimicrobial activity is expected, as the activity is based not only on the different structures of microorganisms but also on their susceptibilities (9). This inhibitory action could be attributed to the phytochemical constituents, since these constituents are well established as antimicrobial agents (10). In the present study, in the agar diffusion assay the methanol and chloroform extracts showed the larger inhibition halo enabling to observe the extraction potential of the solvent employed.

Differences of biological activity between the fractions can be partly explained by quantitative and qualitative variations in the secondary metabolites present in the fractions which are in support with (11) and could be due to the use of different parts of the plants and leading to the extraction of different compounds and with antimicrobial activity. GC-MS technique was used to effect complete separation and identification of the pure compounds in the combined fractions. The presence or absence of functional groups in an organic molecule determines the manner in which that organic molecule will fragment. The structure of the compounds can be deducing by interpretation of various mass peaks in each spectrum. The compounds identified are phthalic acid, butane, methyl palmitate, n-hexadecanoic acid, phytol among others.

CONCLUSIONS

The present study has investigated the efficacy of Calotropis procera which can be considered in folklore or traditional medicine, edible vegetable and animal forage.

The overall results showed that the crude fractions of this plant has an appreciable antibacterial activity on the selected microorganisms.

The chemical compounds present in the plant simplifies that C. procera extracts could maximally serve as alternative to highly rated synthesized

drugs, whose costs are unaffordable by the common man.

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