Characterization and Pharmaceutical Potentials of *KhayaSenegalensis* Seed Oil from Pankshin Plateau State

Alexander O. Edah[*a], Seltim B. Dalmak[b] and Wapwera A. Jidimma[c]

[a]*Department of Chemistry, Faculty of Natural sciences, University of Jos, Jos, Nigeria*

[b]*Department of Chemistry, Faculty of Natural sciences, University of Jos, Jos, Nigeria*

[c]*Department of Microbiology, Faculty of Natural sciences, University of Jos, Jos, Nigeria*

**Corresponding author*

Abstract:- **The fruit of Khayasenegalensis plant, family Meliacease, were collected from Pankshin in the month of November and appropriately identified. The seed oil was extracted using steam distillation method, gas chromatography and mass spectroscopy (GC-MS) was performed, antioxidant analysis was done using DPPH according to Brand-William, Gallic and ascorbic acids served as standard. Anti-microbial activities was determined using Agar diffusion method with Gentamicin 10mg/ml serving as standard. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) were determined with tween 80 as diluent. The GC-MS indicated the presence of urs-12-ene (C_{30}) and dodecanoic acid (C_{12}) etc. the extract does not show appreciable antioxidant properties and the antibacterial analysis shows that the activity of the oil on organism isolate increase with increasing concentration, the oil inhibited Staphylococcus aureus, Salmonella typhi and E.coli . The oil inhibited all the organism isolate at the minimum concentration of 31.25mg/ml. However the oil displayed bactericidal activities on organism isolate throughout the concentration. The effectiveness of the oil on organism isolate justified the claim among the traditional communities for treatment of ailment such as typhoid, skin rashes, boils and abscesses as plausible.**

Keywords: **Khayasenegalensis; GC-MS; Antioxidant; Antimicrobial**

I. INTRODUCTION

Finding healing power in plant is an old idea. People world-over long applied poultice and imbibed infusion of thousands of indigenous plant dating to prehistory.

Human disease management in Nigerian history also provides evidence of the relationships of plants medicine. The medicinal flora in the tropical eco-region has a preponderance of plants that provides raw material for addressing arange of medical disorder and pharmaceutical requirement. Collectively, plants produce a remarkably diverse array of over 5000,000 low molecular mass natural products also known as secondary metabolite. The medicinal value of this secondary metabolite is due to the presence of chemicals substances that produce a definite physiological action on the human body. The important of these include: alkanoids, glucosides, glycosides, steroids, flavonoids, fatty oils, phenols phosphorus and calcium for cell growth, replacement, and body building. The plant, *khayasenegalensis*(*madachi* in Hausa, *Oganwa* in Yoruba, and *Ono* in Igbo), is a savanna tree, easily recognized by its round evergreen crown of dark shinning foliage pinnate leaves and characteristic round capsules. Atree of 30m height 3m girth, with dense crown and short bole covered with dark grey scaly bark. Slash dark pink, bitter yielding gum when wounded. Leaves with 3 – 4(exceptionally 5) pairs of leaflet ,5 – 10cm long by 2.5 – 5 broad, more or less elliptic, rounds obtuse or shortly acuminate at apex; stalks of leaves lets 4mm long

II. MATERIAL AND METHODS

The fruit were open with a sharp knife and the seeds removed. The thin covering on the seeds were peeled off to expose the creamy white seeds. The seeds were sun dried until crisped and then roasted on the earthenware pot on firewood.

The seeds were pounded in a wooden mortal with pestle into smooth paste. Wood ash was soaked in water and stirred to thoroughly ensure mixing and boiled on firewood with intermittent stirring for 3 hours with time the oil floats on top and is skinned off. The oil was heated to remove any residual moisture and filtered and kept in an airtight container for subsequent use.

2.1 Antioxidant Activity

The antioxidant activity (free radical scavenging activity) of the extract on the stable radical 1,1-diphenyl-2picrylhdrazyl (DPPH) was determined according to the method described in (Brand Williams, *et al.,* 1995). The following concentrations of aqueous extract of*Khayasengenelensis* was prepared 500, 250, 125, 62.50, 31.25, 15.62, 7.8125, 3.91, 1.95 and 0.98µg/ml. All the solutions were prepared with methanol. 2mL of each prepared concentration was mixed with 4mL of 50µM DPPH solution in methanol. Experiment was done in triplicate. The mixture was vortexed for 10s to homogenize the mixture and test tubes were incubated for 30mins at room temperature in the dark, after 30mins of incubation the absorbance was measured at 515nm using UV-vis spectrophotometer. Lower the absorbance of the reaction

mixture indicates higher free radical scavenging activity. Gallic acid and rutin were used as standards with the following concentration 100, 50, 25, 12.5, 6.25, 3.125, 1.563, 0.7812, 0.391 and 0.195µM. Blank solution was prepared by mixing 2mL of methanol with 4mL of 50µM DPPH solution in methanol.

The difference in absorbance between the test and the control (DPPH methanol) was calculated and expressed as % scavenging of DPPH radical. The capability to scavenge the DPPH radical was calculated by using the following equation.

$$\% \text{ inhibition} = \frac{\text{ABS control} - \text{sample}}{\text{ABS control}} \times 100$$

Finally, the IC_{50}value, defined as the concentration of the sample leading to 50% reduction of the initial DPPH concentration, was calculated from the separate linear regression of plots of the mean percentage of the antioxidant activity against concentration of the test extract (µg/mL).

2.2 Preparation of culture media

5.0g of Mueller Hinton agar was dissolved in 250ml of water and sterilized by autoclaving at 121^{o}c for 15mins. Cork borer of diameter 4mm was used to bored five holes on the plate that was inoculated with a single strain organism isolate and about 0.2m of diluted extract concentration was introduce into the holes bore with respect to the labels on the plate from the highest to the lowest concentration the plate was allowed to stand for 1 hour to ensure pre diffusion before incubation at 37^{0}c for 24 hours.

2.3 Preparation of khayasenegalensis oil for sensitivity test

5ml of the oil was pipette into a small bottle and 10ml of Tween 80 solution was added to it, the bottle was closed and shaken vigorously to ensure mixing, double serial dilution was done with this to obtain 500mg, 250mg, 125mg, 62.5mg and 31.25mg of the oil concentration respectively, these various concentration was introduced in the plate containing prepared culture media using Mueller Hinton agar. Gentamicin 10mg/ml was used as a standard control. The plate was left for 1 hour at room temperature to allow for the diffusion and then incubated at 37^{o}c for 24 hours. The zones around the holes show inhabitation and was measured in mm using a transparent plastic rule line.

2.4 Minimum Inhibitory Concentration (MIC)

The MIC of the extract was determined using small bottles 5 for each organism isolate for the 500, 250, 125, 62.5, and 31.25mg different concentration o.1ml of E.coli, staphylococcus aureus and Samonella was inoculated into the bottles containing diluted extract of the various concentrations and incubated at 37^{o}c for 24 hours.

2.5 Minimum Bactericidal Concentration (MBC)

The culture media was prepared using Mueller Hinton agar 5.0g of the agar was dissolved in 250ml distilled water and autoclave to sterilized at 121^{0}c for 15mins. At the concentration in which the organism isolate shows positive test (there was no growth in the bottles) small quantity was picked into a small culture media aseptically using aflame ware loop and then incubated at 37^{0}c for 24 hours in the incubator this was done to subculture the isolate

III. RESULTS AND DISCUSSION

Table 1: DppH scavenging activity of aqueous extract of Khayasenegalensis and Gallic acid

The DPPH scavenging activity table 1 was done and the extract does not show appreciable antioxidant activity as compared to Rutin, Gallic and ascorbic acids as standards.

Con(µg/ml)	g/ml	% inhibition of extract	Conc µg/ml		% inhibition Rutin	% inhibition Gallic	% inhibition (vitamin C)
500	0.0005	45.9	100	0.0001	89.27	92.12	75.2
250	0.00025	39.7	50	0.00005	82.20	91.93	81
125	0.000125	35.1	25	0.000025	60.41	92.49	83.6
62.5	6.25E-05	32.6	12.5	1.25E-05	52.43	92.02	85.5
31.25	3.13E-05	30.8	6.25	6.25E-06	46.55	71.58	86.1
15.62	1.56E-05	30.9	3.125	3.13E-06	45.63	53.97	86.6
7.8125	7.81E-06	30.2	1.5625	1.56E-06	43.47	48.39	86.7
3.91	3.91E-06	28.5	0.7812	7.81E-07	44.12	43.67	73.4
1.95	1.95E-06	28.8	0.391	3.91E-0	44.50	40.56	50.7

vitamin C and Rutin IC_{50} =631.0±0.11µg/ml. IC_{50}Rutin = 75.3µg/ml IC_{50} Gallic acid = 47.3µg/ml IC_{50} = 11.3µg/ml

Table 2: Susceptibility of the plant extract (K.senegalensis) on the test organisms zones of inhibition (mm) concentration (mg/ml)

Test organisms	**500**	**250**	**125**	**62.5**	**31.25**	**+VE C**
E-coli	20	19	17.5	15	14	27.5
Salmonella typhi	20	19	18	16	13	25
Staphylococcus aureus	20	19	17	16	15	27.5

Key

- = No zone of inhibition
= Positive control (+ve C) = 10mg/ml

12 min and below = resistances
13 min and 14 min =moderately sensitive
15 min and above = sensitive

Table 3: minimum inhibitory concentration (MIC) ofK.senegalensis on the test organisms

Test organisms	500	250	125	62.5	31.25	MIC
E.coli	-	-	-	-	-	31.25
Salmonella typhi	-	-	-	-	-	31.25
Staphylococcus aereus	-	-	-	-	-	31.25

Key

- = no growth
+ = growth

Table 4 minimum bactericidal concentration (MBC)

Test organisms	500	250	125	62.5	31.25	MBC
E.coli	-	-	-	-	-	31.25
Salmonella typhi	-	-	-	-	-	31.25
Staphylococcus aereus	-	-	-	-	-	31.25

Table 5 GC-MS Results

Compound name	**Chemical formular**	**Molecular weight**	**R-Time**
2-octenal	$C_8H_{14}O$	126	1013
Dodecanoic acid	$C_{12}H_{24}O_2$	200	1570
Tridecanoic acid	$C_{13}H_{26}O_2$	214	1670
2,6,10-trimethyldecane	$C_{15}H_{35}$	212	1320
Tetradecanoic acid	$C_{14}H_{28}O_2$	228	1769
2-methylhexacosane	$C_{27}H_{56}$	380	2641
9,19-cycloergost-24(28)-en-3-ol	$C_{30}H_{50}O$	426	2760
Urs-12-ene	$C_{30}H_{50}$	410	2685
4,22-stigmastadiene-3-one	$C_{29}H_{46}O$	410	2722
2,2,4-trimethyl-3-(3,8,12,16-tetramethyl-heptadeca-3,7,11,15-tertaenyl)-cyclohexanol	$C_{30}H_{52}O$	428	2075

Medicinal plant constitutes an effective source of both traditional and modern medicines, but assessment of antimicrobial potential of the sources is essential.

The present study found a very promising and readily available source (*KhayaSenegalensis*) for treating infections caused by some bacterial. This is particularly significant because drug resistance to human pathogens has been increasing not only in the developing countries but throughout the world to indiscriminate use of antibiotic (Barie 2012).

The seed oil was effective against enteric bacterial such as E.coli, salmonella and staphylococcus aureus from the investigation carried out on the antibacterial activity of the oil (Table2), the oil was found to have higher activity with increasing concentration both on Gram-Negative and Gram-positive bacteria. The minimum inhibitory concentration (MIC) Table 3 was determined with the following concentration 500,250, 125, 62.5 31.25 and result show that, the oil *inhibited E,coli, salmonella typhi and staphylococcus aureus* at the concentration of 31.25mg/ml. Table 4 show the extract kill the organism isolate throughout concentration. Therefore the MBC is 31.25mg/ml.

IV. CONCLUSION

*KhayaSenegalensi*s is widely used in traditional medicine to combat and cure various ailments and found to be rich in secondary metabolites. The presence of Tannins, Saponin, alkaloids, flavonoids and oxalate in the plant may be attributed to their curative properties. The exploitation of these pharmacological properties is imperative because these support the claim among the traditional communities for its potential as therapeutic agents for the treatment of urinary tract infection (UTI), Respiratory Tract Infection (RTI), Typhoid, skin rashes, boil and abscesses.

In addition to traditional claims antioxidants activities of the plant extract was carried out to see if would be effective for managing or preventing Cardiovascular disease, cancer and pre-mature ageing but the result was counterproductive.

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