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PHYTOCHEMICAL AND MICROBIAL SCREENING OF *PARKINSONIA ACULEATA* L. LEAVES

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ABSTRACT

Preliminarily phytochemical and antimicrobial investigation of the crude extract of the leaves of parkinsonia aculeata leaves showed the presence tannin, alkoloids,glycoside,terpenoids flavonoid, terpenes, streriods volatile oil and saponin.The presence of these secondary metabolites indicates the pharmacological property of the plant leaves. The crude ethanolic extract, petroleum ether and chloroform extracts were also found to inhibit pseudomonas aeruginosa, streprococcus faecalis staphylococcus aureus, escherichia coli, and salmonella typhimurium and klebsiella sp. The tin layer chromatography(TLC) revealed four spots, three spots and two spots for ethanol, petroleum ether and chloroform extract respectively using ethyl acetate: hexane solvent mixture. The minimum inhibitory concentrations (MIC) of the crude extracts were determined for the various organisms which ranged between 35 and 50 mg/ml while the minimum bactericidal concentration (MBC) ranged between 45 and 60 mg/ml. parkinsonia aculeata could be a potential source of antimicrobial agents.

Key words: parkinsonia aculeata, phytochemical Screening, antimicrobial activity, minimum bactericidal concentration, minimum inhibitory concentrations

Introduction

History will certainly agree with the fact that, man has used plants to treat common infectious diseases, and some of the traditional medicines are still included as part of the habitual treatment of various maladies [1]; [2] Scientific interest in medicinal plant has burgeoned in recent times due to increased efficiency of new plant derived drugs and rising concerns about the side effects of modern medicine. The continuing emergence of drug resistant organisms and the increasing evolutionary adaptations by pathogenic organisms to commonly used antimicrobials have reduced the efficacy of antimicrobial agents currently in use.

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Therefore, the search for new drugs from novel sources such as plants continues to be necessary [3]. To date, plants continue to be a major source of commercially consumed drugs. Even most synthetic drugs have their origin from natural plant products [4]. Screening of medicinal plants for antimicrobial agents has gained much importance because lately World Health Organization (WHO) is keenly interested in the development and utilization of medicinal plant resources in the traditional system of medicine in the developing countries so as to extend the health care to maximum number of population in these countries [5]. According to World Health Report of Infectious diseases 2000, overcoming antibiotic resistance is the major issue of the WHO for the next millennium. Hence the last decade witnessed an increase in the investigation of plants as a source of human disease management [6].

Parkinsonia aculeata L., is a large spinous shrub or small tree from the family Fabaceae, the common names include Mexican Palo Verde,Jerusalem thorn, or Jellybean tree. It is native to the south western United States (western Texas, southern Arizona), Mexico, the Caribbean, South America south to northern Argentina, and the Galapagos Islands [7]. *Parkinsonia aculeata* can grow to 8 m, although smaller plants are more common. It can be single- or multi-stemmed. The smooth, green stems are slender and tend to droop and zig-zag. Its leaves are quite different to the ferny leaves of the three other prickle bushes *Parkinsonia aculeata* leaves consist of a flat, green leaf stalk up to 300 mm long and 2–3 mm wide with numerous small (4–10 mm) green oblong leaflets staggered along both sides. The leaf base is protected by sharp, recurved spines, 5–15 mm long, which persist in older branches. *Parkinsonia aculeata* flowers are about 20 mm across, with four yellow petals and one erect orange or orange-spotted petal. Seed pods (30–130 mm long) are straight with bulges around seeds and points on both ends, and are straw brown when ripe. They generally contain 1–4 seeds, but occasionally up to 11. Seeds are olive-green to brown and oblong-shaped (10 mm by 4 mm). The roots are generally shallow [8].

All the parts of the plant are known as antipyretic, diaphoretic, and abortifacient (Wealth of India, 1948-66). The alcoholic extract of the aerial part possess CNS depressant activity [9]

Material and Methods

Sample collection

The leaves of the plant were collected in Chancha, Gwadabawa local government area, Sokoto state, the plant was botanically identify in the taxonomy unit of Usmanu Danfodiyo University, Sokoto. while the pathogenic micro organisms were obtained from Usmanu Danfodiyo University teaching Hospital, sokoto, the organisms are as follows with there registration Number: *Klebsiella* sp*(*SB-101), *Escherichia coli* (SB-981),*Pseudomonas aeroginosa* (SB-845), *Salmonella typhimurium (*SB-1014), *Staphylococcus aureus (*MB-123).

Parkinsonia Aculeata L.

Preparation of the Aqueous Extract

Samples (50g) of thoroughly washed fresh *parkinsonia aculeata* leaves were macerated with 100ml sterile distilled water in a Waring blender (Waring International, new Hartford, CT, USA) for 10min. The macerate was first filtered through double-layered muslin cloth and then centrifuged at 4000*g* for 30 min. The supernatant was filtered through Whatmann No.1 filter paper and sterilized at 120^0C for 30 min. These extracts were allowed to cool to room temperature and their p^H was determined just before subjecting it to antibacterial activity assay.

Preparation of Solvent Extracts

The mature leaves were thoroughly washed shade dried and then powdered with the help of waring blender. Twenty-five grams of the powder was filled in the thimble and extracted successively with petroleum ether, chloroform, ethanol and using a Soxhlet extractor for 48h. All the extracts were concentrated using rotary flash evaporator and preserved in airtight bottle prior to the commencement of the analysis. All the extracts were subjected to antibacterial activity assay and phytochemical analysis.

Antibacterial Activity Assay

The four different concentrations of the leaf extracts were tested for antibacterial activity using agar disc diffusion assay according to the method of [10]. The strains of microorganisms obtained were inoculated in conical flask containing 100 ml of nutrient broth. These conical flasks were incubated at 37^0 C for 24 h and were referred to as seeded broth. Media were prepared using Muller Hinton Agar (Himedia), poured on Petri dishes and inoculated with the test organisms from the seeded broth using cotton swabs. Sterile discs of six millimeter width had been impregnated with 20 µl of test extract and introduced onto the upper layer of the seeded agar plate. The plates were incubated overnight at 37^0 C. Antibacterial activity was assigned by measuring the inhibition zone formed around the discs. The experiment was done three times and the mean values were presented. Streptomycin (10µg/disc) and penicillin (10µg/disc) were used as standards.

Thin Layer Chromatography (TLC)

Thin Layer Chromatography (TLC) was used to separate the leaves extcart into different spots on the chromotoplate. The chromatograms developed on the microscope slide, were dried and observed visually for the various leaves components. The developing solvents used are ethyl acetate: Hexane (1:1), mixture. All the corresponding spots were again subjected to antibacterial activity.

The retention factor was calculated using:

R_f =) *Distance move by the substance(cm*

Distance move by the solvent front (cm)

Phytochemical Analysis of Plant Leaves Extract

The methods described by [11] and Trease and Evans [12] with slight modifications were used to test for the presence of the active ingredients in the test sample.

Determination of Minimal Inhibitory Concentration (MIC)

MIC was determined by both agar and broth dilution methods. For broth dilution tests, 0.1ml of standardized suspension of bacteria (106 CFU/ml) was added to each tube containing different concentrations of the active fraction (0-20 µg/ml) and incubated for 24h. at 37^0C. In agar plating method dilutions having 0-20 µg/ml of active fraction was placed in the cups on the inoculated plate and incubated as mentioned above. The lowest concentration of the tube or plate that did not show any visible growth by macroscopic evaluation was considered as the MIC [13]. The data obtained were statistically analyzed using Analysis of Variance (ANOVA), as described by [14].

Determination of Minimum Bactericidal Concentration (MBC)

After culturing the test organisms separately in nutrient broth containing various concentration of the stem bark extract of the plant, the broth was inoculated onto freshlyprepared agar plates to assay for the bactericidal effect. The culture was incubated at 37°C for 24h. The lowest concentration of the plant extract that does not yield any colony growth on the solid medium after the incubation period was regarded as MBC [15].

Results and Discussion

Table 1: Phytochemical analysis of leaves extract using various solvents

Active principle	cholrofom	Ethanolic extract	Petroleum ether	Crude water extract
Steroids	+	+	-	+
Terpenoids	-	+	+	+
Tanin	+	+	+	+
Flavonoids	+	+	+	+
Alkaloids	+	+	+	+
Saponins	+	+	-	+
Glycosides	-	-	+	+
Anthraquinone	-	+	+	-
Volatile oil	+	+	+	+

Table 2: percentage yield of aqueous and organic solvent extract of *parkinsonia aculeata leaves*

Fractions	Weight of the powdered sampled (g)	Weight of the sample extract (g)	Percentage yield %
Ethanol	50g	4.03	8.06
Petroleum ether	50g	2.56	5.12
Chloroform	50g	2.25	4.50
Crude water extract	20g	5.56	27.8

Percentage yield= Weight of the sample extract obtained (g) x 100
Weight of the powdered sampled used (g) 1
Overall yield=8.06+5.12+4.50+27.8= 45.48%

Table 3: TLC Result of ethanol, petroleum ether, and chloroform extract.

Extract	Solvent system	Number of component	Distance of spot(cm)	Solvent front(cm)	R_f value
Ethanol	Ethyl acetate:	4	6.5,	7.0	0.93
	Hexane		5.3	7.0	0.76
	(1:1)		3.2	7.0	0.46
			1.5	7.0	0.21
Petroleum ether	Ethyl acetate:	2	6.5	7.0	0.93
	Hexane		4.0	7.0	0.57
	(1:1)				
Chloroform	Ethyl acetate:	3	6.5	7.0	0.93
	Hexane		5.4	7.0	0.77
	(1:1)		1.7	7.0	0.24

Table 4: Antimicrobial activities of leaves extract.

Sl. No.	**Bacterial strains used**	**Zone of Inhibition in mm**					
		Penicillin	Streptomycin	50mg/ml	100mg/ml	200mg/ml	300mg/ml
1.	*Salmonella typhimurium*	19.80±0.81	18.70±0.35	08.60±0.72	11.80±0.66	12.40±0.58	16.87±0.44
2.	*Pseudomonas aeroginosa*	15.30±0.33	9.90±0.47	07.20±0.48	10.88±0.39	13.96±0.36	17.10±0.54
3.	*Klebsiella sp*	16.10±0.25	11.60±0.71	09.10±0.51	12.64±0.42	13.96±0.33	15.80±0.53
4.	*Escherichia coli*	11.70±0.60	16.10±0.25	07.40±0.54	08.72±0.46	11.08±0.45	14.08±0.71
5.	*Staphylococcus areus*	23.80±0.25	21.40±0.35	08.60±0.61	11.68±0.33	13.37±0.50	16.87±0.58
6.	*Streptococcus faecalis*	19.10±0.50	21.80±0.45	08.10±0.11	11.90±0.55	14.70±0.60	16.20±0.65

Table 5: Minimum inhibitory concentration (MIC) of leaves extract

Organism MIC	**(mg/ml)**
Streprococcus faecalis	40
Pseudomonas aeruginosa	35
Staphylococcus aureus	40
Escherichia coli	45
Salmonella typhimurium	50
Klebsiella sp	45

Table 6: Minimum Bactericidal Concentration (MBC) of leaves extract.

Organism	**(mg/ml**
Streprococcus faecalis	40
Pseudomonas aeruginosa	45
Staphylococcus aureus	45
Escherichia coli	45
Salmonella typhi	50
Klebsiella sp	60

Discussion

Plant based drugs are gaining popularity because of several advantages such as fewer side effect, better patient tolerance, relatively less expensive and acceptance due to a long history of use, especially herbal medicines has provide rational means for the treatment of many diseases that are incurable in other system of medicine.

The results of qualitative phytochemical and antibacterial activity of *parkinsonia aculeata* leaves are presented in table 1 to 6. In this investigation, the plant *leaves* was reported to have maximum studied compounds. Table 1 indicate that both the solvent used gave positive result for alkaloid, flavonoid, volatile oil, tannin, with the absent of saponin and steroid in the petroleum ether fraction while present in all the remaining solvents used, and. Anthraquinone were also found absent in the chloroform and crude water extract, while glycoside was absent in the ethanolic and chloroform extract of the plant leaves. Finally the water crude extract contained all the secondary metabolite analysed with the exception of Anthraquinone, while terpenoids were absent in the chloroform extract but indicating a positive results in all the solvent used. It was reported that several phenolic compounds like tannins present in the cells of plants are potent inhibitors of many hydrolytic enzymes such as proteolytic macerating enzymes used by plant pathogens. Other preformed compounds like saponins also have antifungal properties [16]. Many plants contain non-toxic glycosides that can get hydrolyzed to release phenolics that are toxic to microbial pathogens [16]. Therefore the principle active compounds detected may be responsible for the antibacterial activity of the tested organisms.

Table 2 shows the result of weight of solute extracted from 50g of powdered leaves and percentage yield of the plant crude extract using different solvents, the result indicate that ethanolic extract has the higher percentage yield with 4.03g representing 8.06% followed by the petroleum ether extract having 2.56g with 5.12 % while the chloroform extract has 2.25g with the percentage of 4.50% having the least percentage yield, Which is in contrast with the last crude water extract that has 5.56g representing 27.8%, this is because it does not contain any organic solvent, so it has highest percentage yield in all the four solvents used during the analysis. The overall percentage yield of the extracts has 45.48%. From the results obtained, indicate that ethanol gives a better solvent of

extraction when compared with the petroleum ether and chloroform respectively, while the water extract is the best among all the solvents used.

The results TLC analysis using ethyl acetate: Hexane solvent mixture as shown in table 3 which revealed four spot for ethanol, two spot for petroleum ether and three spot for chloroform extractions.

The results in Table 5 indicate that the MIC of the leaves extract of the plant ranged between 35 and 50 mg/ml. The effect of the plant extract on the MIC for the test microorganisms is in line with the report that microorganisms varied widely in the degree of their susceptibility [16]. An antimicrobial agent with highly active antimicrobial agent gives a low MIC while a low activity against an organism has a high MIC. The minimum bactericidal concentration (MBC) of the leaves extract of the plant ranged between 45 and 60 mg/ml (Table 6). The MIC and BC is normally used to evaluate the efficacy of the agents such as antiseptics, disinfectants and indeed chemotherapeutic agents [16] under standard conditions also support the sensitivity test results.

Conclusion:

Antibiotics provide the main basis for the therapy of bacterial infections. However, the high genetic variability of bacteria enables them to rapidly evade the action of antibiotics by developing antibiotic resistance. Thus, there has been a continuing search for new and more potent antibiotics [17].

This study therefore provided bases to the folkloric use of this plant as a remedy for urinary tract infection, antipyretic, diaphoretic and abortifacient and other infections caused by the pathogens studied as practiced ethnomedically the world over.

Parkinsonia aculeata leaves have showed maximum antibacterial activity and so this plant can be used to discover bioactive natural products that may serve as leads for the development of new pharmaceuticals that address hither unmet therapeutic needs. Such screening of various natural organic compounds and identifying active agents is the need of the hour, because successful prediction of lead molecule and drug like properties at the onset of drug discovery will pay off later in drug development.

Suggestion for further studies, purification and characterization of the phytochemicals (principle active compound) that would be obtained with a view to obtaining useful chemotherapeutic agent.

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