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Antimicrobial activity of the leaf extract and fractions of *Lupinus arboreus*

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The antimicrobial effect of the leaf extract and fractions of *Lupinus arboreus* was investigated. The crude methanol extract (CME) of the dried leaves obtained by 48 h cold maceration was fractionated into n-hexane fraction (HEF), ethyl acetate fraction (EAF), and methanol fraction (MEF); and evaluated using modified agar-well diffusion method. The results showed that the extract and fractions at varying concentrations, exerted strong antimicrobial activity on some of the test organisms. However, a weak activity was observed on the tested fungi-*Aspergillus niger* and *Candida albicans*. Ethyl acetate fraction showed the highest activity on many organisms than extract and other fractions.

Key words: *Lupinus arboreus*, antimicrobial activity, standard drugs, test organisms, extracts and fractions.

INTRODUCTION

The search for novel antimicrobial agents from medicinal plants to combat pathogens has become crucial for avoiding the emergence of untreatable bacterial infections (Bandow et al., 2003; Pfaller et al., 1998). Micro organisms have unfavourable effects on the quality and safety of life. Synthetic chemicals are widely used against these microorganisms. Unfortunately, they develop resistance to many antimicrobial agents. The reason for this high resistance to commonly used antimicrobial agents may not be unconnected with worldwide and indiscriminate use in the environment (Anyim et al., 2010; Mukherjee et al., 2002). In addition, these antimicrobials sometimes cause allergic reaction and immunity suppression. The use of essential oils and plant extracts, therefore, is less damaging in the human health and environment (Isman, 2000; Misra and pavlovstathis, 1997). Plants have provided an arsenal of chemicals to survive attack by a microbial invasion (Martini et al., 2004). Literatures showed that natural products and their derivatives represent more than 50% of the drugs in chemical use with one quarter originating from higher plants (Cragg et al., 1997). *Lupinus arboreus* is easily recognized as a bushy shrub to six feet (1.8 m) tall, with bright yellow sweet-smelling flowers blended with purple and white colours (Pickart and Miller, 1998). Also known as yellow bush, *L. arboreus* occurs as an invasive species in Northern California coastal dunes (Wear, 1998). But in Nigeria, it is planted widely as ornamental plant (Ohadoma et al., 2011). It is highly nutritive and wholesome hence grown for fodder and come close to soybean in protein content (Rachel, 2006). In our previous paper (Ohadoma et al., 2010), i.p LD_{50} of 77.45mg/kg of the methanol leaf extract of *L. arboreus* was reported. This study screened the antimicrobial

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activity of *L. arboreus.*

MATERIALS AND METHODS

Collection and identification of plant

Leaves of *L. arboreus* were collected from Owerri, Imo State, Nigeria and official identification was done by Pharm F. N. Osuala, Head of Pharmacognosy Department, Madonna University where a voucher specimen has been deposited in the herbarium. The leaves were air-dried at room temperature for 28 days and pulverized into fine powder. The powdered leaves (2 kg) was extracted with absolute methanol (Sigma Aldrich, Germany) by cold maceration for 48 h. The mixture was filtered to obtain the methanol extract, which was evaporated using a rotary evaporator (RV 05 Basic IB, IKA Staufen, Germany) and the concentrated methanol extract stored in a refrigerator. Using silica gel column chromatography, dried methanol extract (10 g) was partitioned to yield hexane fraction (HEF), ethyl acetate fraction (EAF) and methanol fraction (MEF). Phytochemical screening of the extract and fractions were carried out (Harbone, 1988).

Test organisms

Pure clinical isolates of *Bacillus subtilis, Pseudomonas aeruginosa, Salmonella paratyphi, Escherichia coli, Staphylococcus aureus, Candida albicans,* and *Aspergillus niger* were obtained from Medical Laboratory Unit of Madonna University Teaching Hospital, Elele, Nigeria.

Determination of sensitivity test and inhibitory zone diameter (IZD)

The modified agar-well diffusion technique was employed (Perez et al, 1990). Each of the test organisms was streaked on the surface of the different sterile sensitivity agar media. Wells were bored on the agar media using sterile cork borer of 6 nm diameter. Exactly 2 drops of the extract prepared as described earlier were accordingly put into the wells and then allowed to stand for 30 min for proper diffusion. Standard drugs (ampicillin 80 µg/ml, tetracycline 40 µg/ml, gentamicin 40 µg/ml, ciprofloxacin 40 µg/ml) were served as control. The plates were then incubated aerobically at 37°C for 24 h.

Determination of minimum inhibitory concentration (MIC)

MIC was determined using the micro broth dilution technique (Irobi et al., 1993). The extract and fractions were incorporated at varying concentrations into nutrient broth respectively containing the test organisms in the test tubes. The control experiment containing the growth medium and each of the test organisms, excluding the extract and fractions were also set. The experiments were incubated at 37°C for 24 h. The lowest concentration of extract and fraction that did not allow microbial growth within the incubation period was taken to be the MIC.

RESULTS

The phytochemical studies showed that methanol extract had the abundance of saponins, glycosides, steroids, terpenes and flavonoids. Resins, protein and reducing sugar occurred in moderate amounts, while alkaloids appeared but in trace amount. Hexane fraction (HEF) contained steroids and terpenes, ethyl acetate fraction (EAF) contained flavonoids and glycosides, while methanol fraction (MEF) contained tannins, saponins and glycosides (Table 1).

Extract and fractions have activity against the test bacteria except *E. coli* and little or no activity on fungi (Table 2). The minimum inhibitory concentration of the extract and fractions on the four organisms that showed sensitivity and IZD are as shown in the Tables 2 to 5.

The results of agar diffusion bioassay of the diluted standard drugs (ampicillin, tetracycline, gentamicin and ciprofloxacin) for MIC determination against the susceptive microorganisms are as shown in the Tables 6, 7, 8, 9 and 10.

DISCUSSION

The solvent extraction of the leaves of *L. arboreus* yielded the crude methanolic extract, while the solvent guided extraction yielded the n-hexane, ethyl acetate and methanolic fractions.

The study revealed the antimicrobial efficacy of *L. arboreus* crude methanolic leaf extract, n-hexane fraction, ethyl acetate fraction and methanolic fraction against clinical isolates of Gram-negative and Gram-positive bacteria responsible for majority of the multidrug resistant infections in Nigeria (Kesah et al., 2003) and *Salmonella* (Akinyemi et al., 2000), urinary tract and asymptomatic genital infections, otitis media and wound infections by *P. aeruginosa* (Oyeka et al., 1995) and *S. aureus* (Akerele et al., 2002), upper respiratory tract infections, periodontal disease and osteomyelitis in children by *Streptococcus* species and *Bacillus* species (Onuba, 1992).

L. arboreus showed appreciable activity against these bacteria using method of agar-well diffusion; *B. subtilis, S. aureus, P. aeruginosa* and *S. paratyphi*, but had weak activity on the test fungi-*A. niger* and *C. albicans*, hence it is broad-spectrum antimicrobial.

Extrapolations from the graph of IZD^2 $(mm)^2$ against log concentration of extract, fractions and standard antimicrobials gave their MIC values. From the result of minimum inhibitory concentration (MIC), it was observed that the greater the IZD produced, the lower the MIC and the more potent the agent. However, the fractions, extract and standard antimicrobials had varying MICs on individual organism. Although, it showed no activity on other organism used in the study, the n-hexane fraction when compared with the other fraction showed the highest activity only on *B. subtilis* (MIC 1.07 mg/ml), followed by ethyl acetate (MIC 1.25 mg/ml), methanolic fraction (MIC 3.98 mg/ml) and crude methanolic extract (MIC 5.62 mg/ml). When the activity of these fractions and extract on *B. subtilis* was compared with those of standard drugs, it was observed that gentamicin (MIC 0.02 µg/ml) showed the highest activity. This was

Table 1. Phytochemical constituents of leaf extract and fractions.

Phytochemical constituent	Extract (12.5 %w/w)	HEF	EAF	MEF
Saponins	+++			+
Glycosides	+++	+++	+++	+
Flavonoids	+++		+++	
Steroids	+++	+++		
Terpenes	+++			
Tannins	++			+
Resins	++			
Protein	++			
Reducing sugar	++			
Alkaloids	+			

Value in parenthesis is the extractive yield. +++ =Abundantly present, ++=moderately present, +=present in trace amount.

Table 2. Sensitivity test and IZD of Isolates.

Parameter	*B. subtilis* IZD	*P. aeruginosa* IZD (Mm)	*S. paratyphi* IZD (Mm)	*E. coli* IZD (mm)	*S. aureus* IZD (mm)	*C. albicans* (mm)	*A. niger* IZD (mm)
n-hexane fraction	14.5	-	-	-	-	-	-
Ethyl acetate fraction	14	11	15	-	15	-	-
Methanol fraction	13	13	16	-	14.5	-	-
Crude methanol extract	16.5	12.5	21	-	11.5	-	-

Where (-) means no inhibition.

Table 3. Result of IZD (mm) and IZD^2 $(mm)^2$ of n-hexane fraction.

Parameter	IZD	IZD^2	IZD	IZD^2	IZD	IZD^2	IZD	IZD^2	IZD	IZD^2
Conc. (mg/ml)	100	-	50	-	25	-	12.5	-	6.25	-
Log concentration	2.00	-	1.6980	-	1.3979	-	1.0961	-	1.7958	-
B. subtilis	5.0	25.0	4.5	20.2	4.0	16.0	3.5	12.2	3.0	9.00
S. aureus	-	-	-	-	-	-	-	-	-	-
P. aeruginosa	-	-	-	-	-	-	-	-	-	-
S. paratyphi	-	-	-	-	-	-	-	-	-	-

Table 4. Result of IZD (mm) and $IZ^2$$(mm)^2$ of ethyl acetate fraction.

Parameter	IZD	IZD^2	IZD	IZD^2	IZD	IZD^2	IZD	IZD^2	IZD	IZD^2
Conc. (mg/ml)	100	-	50	-	25	-	12.5	-	6.25	-
Log concentration	2.00	-	1.6980	-	1.3979	-	1.0961	-	1.7958	-
B. subtilis	9.0	81	6.0	36.0	4.5	20.2	4.0	16	3.5	12.2
S. aureus	8.0	64	5.0	25.0	4.0	16.0	-	-	-	-
P. aeruginosa	4.5	20.1	4.0	16.0	3.0	9.0	-	-	-	-
S. paratyphi	4.5	20.2	3.5	12.2	3.0	9.0	-	-	-	-

followed by Ciprofloxacin (0.02 µg/ml), then tetracycline (MIC1.33 µg/ml) and finally ampicillin (MIC 5.62 µg/ml) upon the high stock concentration. Ethyl acetate fraction showed the highest activity on the other organisms: *S. aureus* (MIC 6.3 mg/ml), *P. aeruginosa* and *S. paratyphi* (MIC 9.4 mg/ml for both). The methanolic fraction showed least activity on *S. paratyphi* (MIC 21.13 mg/ml), but showed the highest activity on both *B. subtilis* (MIC 8.81

Table 5. Result of IZD (mm) and IZD^2 $(mm)^2$ of methanol fraction.

Parameter	IZD	IZD^2	IZD	IZD^2	IZD	IZD^2	IZD	IZD^2	IZD	IZD^2
Conc. (mg/ml)	100	-	50	-	25	-	12.5	-	6.25	-
Log concentration	2.00	-	1.6980	-	1.3979	-	1.0989	-	0.7958	-
B. subtilis	4.0	16.0	3.5	12.2	3.0	9.0	3.0	9.0	-	-
S. aureus	6.0	36.0	5.0	25.0	4.0	16.0	-	-	-	-
P. aeruginosa	6.0	36.0	3.0	9.0	-	-	-	-	-	-
S. paratyphi	4.0	16.0	3.0	9.0	-	-	-	-	-	-

Table 6. Result of IZD (mm) and IZD^2 $(mm)^2$ of crude methanol extract.

Parameter	IZD	IZD^2	IZD	IZD^2	IZD	IZD^2	IZD	IZD^2	IZD	IZD^2
Conc. (mg/ml)	100	-	50	-	25	-	12.5	-	6.25	-
Log concentration	2.0	-	1.6980	-	1.3979	-	1.0989	-	0.7958	-
B. subtilis	10	100	7.5	56.2	5.0	25.0	3.5	12.2	2.0	4.0
S. aureus	12	144	8.0	64.0	6.0	36.0	4.4	19.3	2.0	4.0
P. aeruginosa	16	256	11.5	132.2	9.0	81.0	6.0	36.0	3.0	9.0
S. paratyphi	14	196	9.0	81.0	8.0	64.0	5.5	30.2	2.5	6.2

Table 7. Result of IZD (mm) and IZD^2 $(mm)^2$ of ampicillin (concentration of stock = 80 µg/ml).

Parameter	IZD	IZD^2	IZD	IZD^2	IZD	IZD^2	IZD	IZD^2	IZD	IZD^2
Conc. (µg/ml)	80.00	-	40.00	-	20.00	-	10.00	-	5.00	-
Log concentration	1.9030	-	1.06020	-	1.3010	-	10.0	-	0.6989	-
B. subtilis	9	81	6	36	5	25	3	9	-	-
S. aureus	20	400	18	324	16	256	15	225	12	144
P. aeruginosa	8	64	6	36	4	16	-	-	-	-
S. paratyphi	9	81	6	36	-	-	-	-	-	-

Table 8. Result of IZD(mm) and IZD^2(mm) of tetracycline (concentration of stock = 40 µg/ml).

Parameter	IZD	IZD^2	IZD	IZD^2	IZD	IZD^2	IZD	IZD^2	IZD	IZ^2
Conc. (µg/ml)	40	-	20	-	10	-	5	-	2.5	-
Log concentration	1.6020	-	1.3010	-	1.00	-	0.6989	-	0.379	-
B. subtilis	11.00	121.00	10	100	9	81	6	36	5	2
S. aureus	-	-	-	-	-	-	-	-	-	-
P. aeruginosa	16.00	256.00	11	121	10	100	7	49	-	-
S. paratyphi	17.00	289.00	16	256	11	121	10	100	6	36

Table 9. Result of IZD (mm) and IZD^2 $(mm)^2$ of gentamicin (concentration of stock = 40 µg/ml).

Parameter	IZD	IZD^2	IZD	IZD^2	IZD	IZD^2	IZD	IZD^2	IZD	IZD^2
Conc. (µg/ml)	40	-	20	-	10	-	5	-	2.5	-
Log concentration	1.6020	-	1.3010	-	1.00	-	0.6989	-	0.379	-
B. subtilis	26	676	22	484	21	441	20	400	18	334
S. aureus	26	676	25	625	24	576	21	441	18	334
P. aeruginosa	22	484	16	256	14	196	10	100	6.0	36
S. paratyphi	22	400	18	334	16	256	14	196	12.0	144

Table 10. Result of IZD (mm) and IZD^2 $(mm)^2$ of ciprofloxacin (concentration of stock = 40 μg/ml).

Parameter	IZD	IZD^2	IZD	IZD^2	IZD	IZD^2	IZD	IZD^2	IZD	IZD^2
Conc. (μg/ml)	40	-	20	-	10	-	5	-	2.5	-
Log concentration	1.6020	-	1.3010	-	1.00	-	0.6989	-	0.379	-
B. subtilis	22	484	19	381	18	334	16	256	11	121
S. aureus	28	784	26	675	21	441	16	256	15	225
P. aeruginosa	25	625	16	256	9	81	7	49	-	-
S. paratyphi	25	625	21	441	18	334	17	289	16	256

mg/ml) when compared with crude methanolic extract on *B. subtilis* (1.07 mg/ml) and *S. aureus* (MIC 14.12 mg/ml). It also had least activity on *P. aeruginosa* (MIC 39.8 mg/ml) when compared with MIC (22.38 mg/ml) of crude methanolic extract. The ethyl acetate fraction had equal activity on *P. aeruginosa* and *S. paratyphi*. Therefore, the relative low MIC of ethyl acetate fraction on bacteria stems from the fact that it extracts the saponins content of plant leaves which is claimed to possess antibacterial property (Trease and Evans, 2004).

The standard antimicrobials showed very good activity against all the tested organisms with the exception of tetracycline that showed no activity on *S. aureus*. Meanwhile, it suffixes to say that *L. arboreus* leaf extract and fraction (except n-hexane fraction) had broader spectrum of activity than tetracycline on the tested organisms. The greater activity or potency observed with the use of higher dilution (lowest concentration) of the standard antibiotics when compared with the crude extract and fractions is due to their high purity level thus devoid of impurities or contaminant that may antagonized its activities unlike the plant sample extract and fractions.

The Gram-positive bacteria were more susceptible to plant extract, fractions and standard drugs than the Gram-negative bacteria (even the resistance by *E. coli*). This finding agreed with the susceptibility of the microbes to different plant extracts reported by the researchers (Elastal et al., 2003). This could be explained by the fact that the cell wall of Gram-positive bacteria is less complex and lack the natural sieve effect against large molecules (Hawkey, 1998; Geuld and Booker, 2000).

The individual fractions of sample and crude extract showed no activity on *E. coli* and weak activity against the tested fungi (*C. albicans* and *A. niger*). The high content of saponin and tannin (Ohadoma et al., 2010) could be the basis of its antimicrobial action which is in accordance with the claim that plants rich in saponins and tannins have antimicrobial property (Trease and Evans, 2004). Flavonoids which are present could be very useful antioxidant suggesting the plant importance in the prevention and treatment of tumour (Leslie, 1996).

Conclusion

L. arboreus leaf extracts and fractions have exhibited broad spectrum of activity against certain bacteria. In view of this, more study is needed in the areas of isolation, purification and identification of specific constituent with the antimicrobial property as this will help curb the menace of bacterial resistance in chemotherapy and to enhance the exploration of medicinal properties of ethnobotanicals.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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