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Antimicrobial Assay and GC-MS Analysis of Leaves Extracts Medicinal Plant *Senna hirsuta* (L.).

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ABSTRACT

The antimicrobial assay and gas chromatography mass spectrometry (GC-MS) analyses of *Senna hirsuta* leaves extracts in two different solvents polarity that is *n*-hexane and dichloromethane were evaluated against bacterial and fungal namely *Escherichia coli*, *Salmonella typhi*, *Candida albicans* and *Fusarium oxysporum*. The *n*-hexane extract exhibited significant antibacterial and antifungal activities against the pathogen and not found for dichloromethane extract. The moderate antimicrobial activity found at 60% concentration of *n*-hexane extract with zone of inhibition about 9.6 mm in diameter. Chemical constituents of the *n*-hexane extract was separated and identified by means of GC-MS analyses permitted the identification of 9 constituents. The main components of the *n*-hexane extract were *citronellal* (10.82%), ar-turmerone (20.71 %), tumerone (18.75 %), hexadecanoate acid (3.99%), phytol (19.47 %), oxacycloheptadec-8-en-2-one (5.34 %) and isomer of oxacycloheptadec-8-en-2-one (5.28%).

Keywords: *Senna hirsuta* (L.); antimicrobial; GC-MS, *Escherichia coli*, *Salmonella typhi*, *Candida albicans* and *Fusarium oxysporum*

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INTRODUCTION

Antimicrobial resistance (AMR) is one of the most urgent and serious public health problems that use a significant load in mortality. This happens when microorganisms such as fungi, bacteria, and viruses change when they are treated to antimicrobial drugs [1-6]. Irrational use of antibiotic, and antibiotics abused in developing countries can increase the problem of antimicrobial resistance [7-9]. Some antibiotics have been identified resistance against bacteria such as penicillin, tetracycline, methicillin, erythromycin, etc [10]. Therefore, it is necessary to find other alternative medicine to treat infectious diseases. The use of and search for drugs derived from plants have been accelerated in recent years [11]. Medicinal plants such *S. hirsuta* might represent an alternative treatment in cases of infectious diseases. *S. hirsuta* is a medicinal plant of Leguminosae family and mainly distributed in tropical region [12]. Research on antimicrobial and GC-MS analysis of fresh fruit, leaf, entire plant extracts of *S. hirsuta* have been reported [13-16], but none studies on antimicrobial assay and component analysis of bioactive compounds derived from their *n*-hexane and dichloromethane extracts.

RESULTS AND DISCUSSION

The *n*-hexane and dichloromethane leaves extracts of *S. hirsuta* together with (chloramphenicol and Ketoconazole, as a positive control) and (DMSO as a negative control) were assayed *in vitro* against microbes i.e. *E. coli, S. typhi, C. albicans* and *F. oxysporum*. The *n*-hexane extract showed varying degree of inhibition against the tested bacteria and fungi while no activity was noticed on the dichloromethane extract. Biological activity or zone of inhibition (ZOI) of these extracts against these microbes was presented in Table 1.

Table 1. Average of zone of inhibitions (mm) of *n*-hexane and dichloromethane extracts of *S. hirsuta* against *E. coli, S. typhi, C. albicans* and *F. Oxysporum*

Extracts and control concentration (%)	Zone of inhibition (mm)			
	E. coli	***S. typhi***	***C. albicans***	***F. oxysporum***
n-hexane (40%)	8.3	4.0	2.3	6.7
n-hexane (60%)	9.6	3.6	2.0	8.1
Dichloromethane (40%)	0.0	0.0	0.0	0.0
Dichloromethane (60%)	0.0	0.0	0.0	0.0
Antibacterial Chloramphenicol (0.1%) (Control +)	13.6	4.0	-	-
Antifungal Ketoconazole (2%) (Control +)	-	-	12.5	13.7
DMSO (Control -)	0.0	0.0	0.0	0.0

All concentrations of *n*-hexane extracts showed antimicrobial activity against pathogen bacteria and fungi. The *n*-hexane extract (60%) has shown better antibacterial and antifungal efficacies in inhibiting the growth of pathogens investigated compare to *n*-hexane (40%) with zone of inhibitions 9.6 (mm) for bacteria and 8.1 for fungi which is categorized as a moderate inhibitions. The negative control results (DMSO 99%) did not show any inhibitory zone against pathogens investigated, so it can be said that the results were not influenced by 99% DMSO solvent. *N*-hexane of *S. hirsuta* extract was checked for its chemical profile with GC-MS to find out which compound is responsible for those biological activities (Figure 1).

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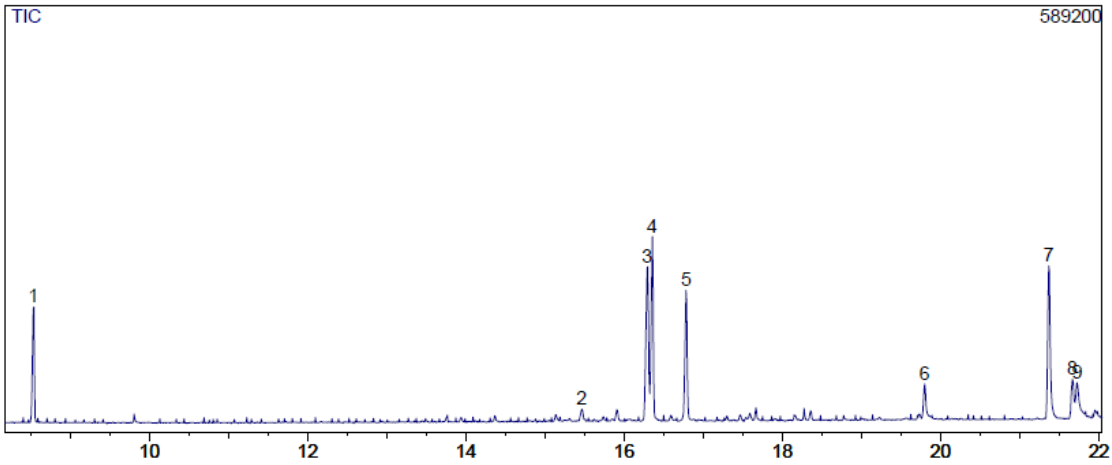

Figure 1. Typical GC-MS spectrum of *n*-hexane extract of *S. Hirsuta*

The chromatogram of *n*-hexane extract of *S. hirsuta* clearly confirmed the presence of 9 compounds with different retention times and percentage as concisely depicted by Table 2.

Table 2. Chemical composition in *n*-hexane extract of *S. Hirsuta*

Peak	RT (min)	Peak Area (%)	MW	Molecular Formula	Name of Compounds
1	8.53	10.82	154	$C_{10}H_{18}O$	Citronellal
2	15.46	1.55	132	-	"Not identified"
3	16.29	20.71	216	$C_{15}H_{20}O$	Ar-Turmerone
4	16.35	18.75	218	$C_{15}H_{22}O$	Tumerone
5	16.78	14.10	218	$C_{15}H_{22}O$	Isomer of tumerone
6	19.80	3.99	256	$C_{16}H_{32}O_2$	Hexadecanoate acid
7	21.37	19.47	296	$C_{20}H_{40}O$	Phytol
8	21.66	5.34	252	$C_{16}H_{28}O_2$	Oxacycloheptadec-8-en-2-one
9	21.72	5.28	252	$C_{16}H_{28}O_2$	Isomer Oxacycloheptadec-8-en-2-one

Note: RT= Retention Time, MW=molecular weight

Table 2 showed that the *n*-hexane extract of *S. hirsuta* had three major components that are ar-turmerone (20.71%), phytol (19.47%), tumerone (18.75%) and citronellal (10.82%). Previous studies reported that hydrodistillation of volatile oil from *S. hirsuta* gave the two main components namely (E)-phytol (30.8 %) and pentadecanal (21.7 %) [16]. Display of antibacterial and antifungal activities of *n*-hexane extract could be due to the array of secondary metabolites such as ar-tumerone, tumerone, phytol and citronellal. Ar-turmerone is a sesquiterpenoid which was isolated from *Curcuma soloensis* Val. is a family plant Zingiberaceae[17]. The sesquiterpenoid exhibited antibacterial and antifungal activities [17-19]. Phytol has been reported giving antibacterial property against *Pseudomonas aeruginosa* [20], as a novel surface disinfectant [21], as a Drug against Neglected Tropical Disease Schistosomiasis Mansoni [22], and antimicrobial [23]. Citronellal oil from *Cymbopogon nardus* has been reported showed antimicrobial against *S. aureus* and *C. albicans* [24]. The GC-MS analysis of dichloromethane extract of *S. hirsuta* gave neophytadiene (23.98%), 3,7,11,15-tetramethyl-2-hexadecene (3.70%), 3,7,11,15-tetramethyl -2-hexadecen-1-ol (4.52%), palmitic acid (8.94%), stearic acid (2.79), and others unidentified compounds.

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MATERIALS AND METHODS

Plant Materials

The healthy leaves of *Senna hirsuta* were obtained from Mr. Rusa Suta farm in Peresak, Narmada, West Nusa Tenggara, and identified by Mr. Gde Mertha the Faculty of Teacher Training and Education, University of Mataram, Indonesia. The sample was shade dried for 7 days to prevent photolysis. The dry leaves were blended to powder with a homogeneous size for extraction process.

Extraction

The powder was soaked in a non-polar *n*-hexane solvent for 24 hours. After 24 hours, the mixture was filtered to obtain *n*-hexane filtrate and insoluble material. The *n*-hexane filtrate was evaporated using a rotary evaporator to obtain *n*-hexane extract. The insoluble material was further soaked in dichloromethane for 24 hours to afford dichloromethane extracts.

Test microbial isolates

Common clinical microbe species includes: *E. coli*, *S. typhi*, and *C. albicans* were obtained from Balai Laboratorium Pengujian dan Kalibrasi, RSUD Provinsi NTB and *F. oxysporum* was collected from Faculty of Agriculture, University of Mataram.

Antimicrobial assay

The antibacterial assay of leaves extracts was performed by the agar diffusion method against *E. coli* and *S. typhi*. A total of 0.15 ml of bacterial suspension with a density of 10^8 (cells/ml) was spread on Nutrient Agar media. The four wells with a diameter of 9 mm were made in each petri dish. Each well was filled with 100 µl (0.1 ml) extract concentration of 40% and 60%,$^{w}/_{v}$, positive control for bacteria (Chloramphenicol 0.1% $^{w}/_{v}$), negative control (DMSO 99%), incubated at 37^{o}C. Observations were made at 48 hours incubation time. Zone of inhibition (ZOI) was observed and measured in mm. The antifungal assay was performed in similar manner of antibacterial assay using *C. albicans* and *F. oxysporum* isolates on Potatoe Dextrose Agar, positive control for fungus (Ketoconazole 2%).

GC-MS analysis

N-hexane extract was analyzed using GC-MS QP2010 system, capillary column model Rx-1 ms 100% dimethyl polysiloxane, length 30 m, diameter 0.25 mm and thickness 0.25 µm. Column oven temperature is at 40^{o}C, injection temperature 260^{o}C. Split injection mode with a ratio of 51.0. The carrier gas is pressure helium 10. The mass spectrum of unknown components was compare with spectrum of the known components stored in the Wiley 7 software library on the GC-MS QP2010.

CONCLUSIONS

The *n*-hexane leaves extract of *S. hirsuta* showed antimicrobial property against the growth of *Escherichia coli*, *Salmonella typhi*, *Candida albicans* and *Fusarium oxysporum*. Ar-turmerone, tumerone, phytol, and citronellal presumably were responsible for this biological activity.

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