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Full Length Research Paper

Phytoconstituent screening and antimicrobial principles of leaf extracts of two variants of *Morus alba* (S_{30} and S_{54})

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Efficacies of the two variants of *Morus alba* locally grown in Forestry Research Institute of Nigeria, were investigated following the recommended procedure of analysis. Phytochemical, proximate and mineral compositions of *M. alba* leaves were determined using a standard procedure. Antimicrobial properties of leaf extracts of *M. alba* were also investigated against laboratory standards of both bacteria and fungi using the disc diffusion method. Phytochemical screening showed the presence of saponins, alkaloids, tannins, oxalate and flavonoids. Trypsin inhibitors, phytate and phenolic compounds were also present in appreciable quantity. Proximate analysis revealed the presence of crude protein, crude fat and crude fibre which is an indication of the plant's nutritional value and could find an application in feed suppliments. The moisture content of *M. alba* (S_{30}- 4.350% and S_{54}- 1.050%) is indicative of its non-perishability, therefore could be stored for a long period of time. Minerals are needed for some physiological functions and some of the minerals are supplied from external sources. *M. alba* (S_{30} and S_{54}) showed the presence of potassium, calcium, phosporus, zinc and magnesium. The minerals investigated revealed that the plant has low mineral content with the exception of potassium (S_{30}- 32.650 mg/100 mg and S_{54}- 31.400 mg/100 mg) which was high. The antibacterial activity was evaluated against microbes by detecting zones of inhibition and minimum inhibitory concentration (MIC). The zones of inhibition were compared with standard antibiotic discs of bacitracin for bacteria and streptomycin for fungi. The MIC values of the cold water extracts of the plant were low when compared with both hot water extracts and ethanol (99.7%) extracts. This is an indication of high potency and most suitable to combat nocosomial infection. *M. alba* extracts could find applications in the treatment of infections as indicated by the result of the antimicrobial screening and the whole plants can also be used in feed compounding for domestic animals.

Key words: *Morus alba*, antimicrobial, phytoconstituents, medicine, organisms.

INTRODUCTION

Plants are the richest resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs (Hammer et al., 1999).

Medicinal plants are of great economical value all over the world. Nature has bestowed on us a very rich botanical wealth and a large number of diverse types of plants grown in different parts of the country. Nigeria is rich in all the 3 levels of biodiversity, namely species diversity, genetic diversity and habitat diversity. Herbal medicine is still the mainstay of about 75 to 80% of the whole population, and the major part of traditional therapy involves the use of plant extract and their active constituents (Akerele, 1993).

In Nigeria, thousands of species are known to have

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medicinal value and the use of different parts of several medicinal plants to cure specific ailments has been in vogue since ancient times (Lawal et al., 2010). Medicinal plants also represent a rich source of antimicrobial agents. Thus, interest has revived recently in the investigation of medicinal plants to identify novel active phytochemicals that might lead to new classes of microbial drug development. The entire plant source or different parts which include root, leaf, seed, stem, flower, fruit, twigs exudates and modified plant organs can be used to identify potential active principles for various ailments either as a whole plant, crude extract, aqueous or organic extracts (Newsman and Crag, 2007; Dev, 2010; Pavithra et al., 2010). Thus, in a bid to further utilize and not only limiting the use of *M. alba* in sericulture practices, the antimicrobial potential was investigated.

Morus is a genus of flowering plants in the family *Moraceae*. The white mulberry (*M. alba*) is a short-lived, fast-growing, small to medium sized mulberry tree, which grows to 10 to 20 m tall. They are native to warm temperate and subtropical regions of Asia, Africa, Europe and the Americans, with the majority of the species native to Asia, most especially China. It is used for feeding silkworm (*Bombyx mori* L.), its dried leaves have been consumed as herb-tea beverage and food supplements. *M. alba* have been a rich food source, and the leaf extracts of *M. alba* are used for anticancer and antioxidants properties (Benalla et al., 2010; Naowaratwattana et al., 2010). A variety of medicinal properties have been attributed to the different parts of the mulberry plant (Datta, 2000), leaves are also dried and used in infusions in Asia.

Forestry Research Institute of Nigeria (FRIN) has eight varieties of *M. alba* in her sericulture plantation. Laboratory investigations have been carried out on the antimicrobial activities of some of the varieties of *M. alba*. Tirupathi et al. (2011) investigated the antimicrobial properties of *M. alba;* S_{26} and S_{36}, while Manjula and Shubha (2011) also screened the activities of total soluble protein of two varieties of M. alba; S_{13} and M_5 on some pathogenic bacteria. Literature review on *M. alba* S_{30} and S_{54} revealed that no previous phytochemical and pharmacological investigations have been reported.

This study was, therefore, carried out to evaluate the antimicrobial effect of cold water, hot water and ethanol (99.7% v/v) extracts of the leaves of the plant on some selected pathogens so as to establish a scientific basis for its use in treatment of conditions related to bacterial and fungal infections.

MATERIALS AND METHODS

Plant source

M. alba leaves (variants S_{30} and S_{54}) were collected from Herbal Garden, Forestry Research Institute of Nigeria and taken to herbarium section for identification. The plant samples were dried and ground into powder using a laboratory milling machine and then kept away from moisture prior to extraction. Analytical grades of ethanol (99.7% v/v), sulphuric acid, methanol, sodium chloride, ferric chloride and ammonium chloride were all purchased from an authorised dealer.

Extraction and recovery

The processed plant materials (S_{30} and S_{54}) were extracted with cold and hot distilled water and absolute ethanol (99.7% v/v) with constant agitation at 300 rpm for 30 min. The process was repeated three times to exhaustively extract the bioactive materials and then concentrated using rotary evaporator to obtain crude extracts free of the extracting solvents. The crude extracts were then stored under 4°C until required for analysis.

Phytochemical, mineral and proximate analysis

The crude extracts were phytochemically screened using Brain and Turner (1975) methods. Mulberry samples were analyzed for moisture, ash, crude protein, crude fiber and crude fat content using the methods described by AOAC (1990). The mineral contents of calcium, phosphorus, zinc, potassium and magnesium were determined using Ilelaboye and Pikuda (2009) method (Table 3).

Antimicrobial screening

Test organisms

Standard strains of bacteria and fungi used for the work were *Escherichia coli*, *Pseudomonas aeruginosa*, *Neisseria gonorrheae and Proteus vulgaricus* for Gram negative bacteria and *Staphylococcus aureus* and *Streptococcus faecium* for Gram positive bacteria. Fungal isolates; *Aspergillus tamari*, *Aspergillus niger, Fusarium oxysporum* and *Penicillium oxalicum*. All strains were standards obtained from the Microbiology Laboratory of the Institute of Agricultural Research and Training, Obafemi Awolowo University, Ibadan, Nigeria. All bacterial strains were cultivated in nutrient agar (NA) medium, and incubated at 37°C for 24 h, while fungi were cultivated in potato dextrose agar (PDA). These were used for the microbial activity using the disc diffusion assay method.

Antimicrobial activity test by disc diffusion method

Antimicrobial activity of the crude leaves extracts of *M. alba* (S_{30} and S_{54}) were evaluated by the paper disc diffusion method following a procedure used by Brantner and Grein (1994) and modified by Ali et al. (1997). Paper discs impregnated with 20 µl of a solution of 10 mg/ml of bacitracin (for bacteria) and streptomycin (for fungi) as standard antibiotics were used for comparison. Antimicrobial activity was determined by the measurement of zone of inhibition around each paper disc. Three replicate trials were conducted against each organism.

Determination of minimum inhibitory concentration (MIC)

Serial tube dilution technique (Iwaki et al., 2006; Khan et al., 2007) was used to determine MIC of the extracts against Gram positive and Gram negative bacteria and fungi. The procedure was repeated on the test organisms using the standard antibiotics (bacitracin for bacteria and streptomycin for fungal isolates). The minimum inhibitory concentration (MIC) of the extracts was determined for each of the test organisms in triplicates. Tubes

containing bacterial cultures were then incubated at 37°C for 24 h, while tubes containing fungal spore cultures were incubated for 48 h at room temperature (30 to 35°C). After incubation, the tubes were then examined for microbial growth by observing turbidity.

RESULTS AND DISCUSSION

Phytochemical, proximate and mineral compositions of the two variants of *M. alba*

In the present study, the two variants of *M. alba* (S_{30} and S_{54}) used showed appreciable level of all the phytochemicals analyzed. Saponins, phenolic compounds, flavonoids and tannins were all present in the two variants of *M. alba* with saponins having the highest quantity (S_{30}: 4.905 ± 0.04% and S_{54}: 4.820 ± 0.03%) and closely followed by tannins (S_{30}: 3.100 ± 0.00% and S_{54}: 2.665 ± 0.01%) as shown in Table 1. Though, alkaloids, phenolic compounds and flavonoids were present in limited amount. Each of this group of compounds has been reported to possess antimicrobial activity (Ragasa et al., 2005; Cuhnie and Lamb, 2006; Soetan et al., 2006) and reportedly exert their effects by affecting the cell membrane integrity of the bacteria (Hendrich, 2006; Trombetta et al., 2005; Killeen et al., 1998).

Trypsin inhibitors, phytate and oxalate were some of the antinutrients analysed for and showed, they were present in varying levels (Table 1). Oxalate can form metal-complex with some of the trace metals essential for body functions, thereby making them unavailable for enzymatic activities and other metabolic activities. Phytate has been found to cause indigestion of food and flatulence (Maynard, 1997). Though, these antinutrients can easily be reduced to tolerable limits by proper simple processing techniques, such as soaking, cooking and frying (Ekpo et al., 2004), but care must be taken if it were to be used as food suppliments or herbal tea as practiced in China.

The plants were unusually low in crude protein, crude fat and crude fibre as indicated in Table 2 which might actually limit its use as food suppliments. Though, the protein content is relatively low but it can contribute to the proper functioning of hormones which controls a variety of body functions, such as growth, repair and maintenance of body protein (Mau et al., 1999). The low moisture contents of 4.350 ± 0.78% for S_{30} and 1.050 ± 0.07% for S_{54} will confer long shelf life to the plants before cultivation or consumption.

Minerals are needed by the body for some physiological functions. A meal low in calcium can result into ricket and calcification of bone. Distorted enzymatic activity and poor electrolyte balance of the blood fluid are related to inadequate Na, K, Mg and Zn; as they are the most required elements of living cells. The two variants of *M. alba* were low in the minerals analyzed for which could seriously limit their uses as food suppliments. Only potassium (S_{30}: 32.650 ± 0.07 mg/100 g and S_{54}: 31.400± 0.00 mg/100 g) was present in an appreciable level which which signified that the plant could be used in potassium-deficient food.

Table 1. Phytochemical screening of *M. alba*.

Phytochemical constituent	Percentage (%)	
	S_{30}	S_{54}
Saponins	4.905 ± 0.04	4.820 ± 0.03
Phenolic compounds	2.500 ± 0.00	2.605 ± 0.01
Alkaloids	0.895 ± 0.01	0.910 ± 0.00
Trypsin inhibitors	1.420 ± 0.00	1.335 ± 0.02
Flavonoids	2.600 ± 0.00	2.310 ± 0.01
Tannins	3.100 ± 0.00	2.665 ± 0.01
Phytate	3.100 ± 0.00	3.300 ± 0.14
Oxalate	2.900 ± 0.00	2.710 ± 0.04

Tests were done in triplicates and values were expressed as mean in %.

Table 2. Proximate analysis of *M. alba*.

Parameter	Composition (%)	
	S_{30}	S_{54}
Crude protein	1.710 ± 0.01	1.750 ± 0.00
Crude fat	0.070 ± 0.00	0.100 ± 0.00
Crude fibre	0.565 ± 0.02	0.700 ± 0.00
Ash content	6.725 ± 0.04	6.450 ± 0.64
Moisture content	4.350 ± 0.78	1.050 ± 0.07
Dry matter	95.650 ± 0.78	98.950 ± 0.07

Tests were done in triplicates and values were expressed as mean in %.

Table 3. Mineral compositions of *M. alba*.

Parameter	Contents (mg/100 g)	
	S_{30}	S_{54}
Calcium	1.655 ± 0.01	1.570 ± 0.00
Phosphorus	8.200 ± 0.14	8.500 ± 0.14
Zinc	2.625 ± 0.04	2.475 ± 0.01
Potassium	32.650 ± 0.07	31.400 ± 0.00
Magnesium	1.665 ± 0.07	1.725 ± 0.01

Tests were done in triplicates and values expressed as mean in %.

Antimicrobial activities and minimum inhibitory concentration (MIC) of the extracts

Plants have been used as alternative medicine and tend to be safer than the chemical-based medicine. In the present study, *M. alba*, which basically serve as food for silkworm (*B. mori* L.) in Sericulture Unit, Forestry Research Institute of Nigeria, possessed some antimicrobial properties.

Table 4. Antimicrobial activity of ethanolic extracts of *M. alba* (S_{30} and S_{54}).

S/N	Isolate used	Zone of inhibition (mm)		
		S_{30}	S_{54}	Control*
A	*S. aureus*	07	07	14
B	*P. aeruginosa*	08	10	17
C	*S. faecium*	07	10	16
D	*E. coli*	07	07	21
E	*N. gonorrheae*	06	05	24
F	*P. vulgaricus*	08	07	12
G	*A. niger*	04	04	09
H	*A. tamari*	04	05	14
I	*F. oxysporum*	03	04	12
J	*P. oxalicum*	04	04	18

*Bacitracin and streptomycin as standard antibiotics

Table 5. Minimum inhibitory concentration (MIC) µg/ml of *M. alba* leave extracts.

Organism isolate	MIC (µg/ml)				Organism isolate	MIC (µg/ml)			
	Cold H_2O	Hot H_2O	Ethanol	Control*		Cold H_2O	Hot H_2O	Ethanol	Control*
	S_{30} variant of *M. alba*					S_{54} variant of *M. alba*			
S. aureus	600	600	850	700	*S. aureus*	600	650	900	710
P. aeruginosa	450	600	1000	720	*P. aeruginosa*	500	600	1000	700
S. faecium	500	750	1000	800	*S. faecium*	500	800	1100	750
E. coli	550	700	1000	800	*E. coli*	500	850	1000	800
N. gonorrheae	400	600	800	850	*N. gonorrheae*	450	650	850	800
P. vulgaricus	500	550	800	700	*P. vulgaricus*	450	600	850	700
A. niger	50	60	70	60	*A. niger*	50	60	75	60
A. tamari	40	60	70	60	*A. tamari*	45	55	75	60
F. oxysporum	40	65	80	55	*F. oxysporum*	40	70	85	50
P. oxalicum	50	70	70	60	*P. oxalicum*	50	65	70	60

*Bacitracin and streptomycin as standard antibiotics.

Table 4 showed that S_{54} of *M. alba* showed more activities against some of the microorganisms screened with the extracts. It showed more activity against *P. aureginosa* and *S. faecium* with zones of inhibition of 10 mm each. Generally, the antimicrobial activities of S_{30} and S_{54} were low when compared with the control, but the extracts could still be potent against some human pathogenic organisms.

The test organisms used in this study are associated with various forms of human infections. From a clinical point of view, *E. coli* causes septicemias and can infect the gall bladder, meninges, surgical wounds, skin lesions and the lungs, especially in debilitate and immuno-deficient patients (Black, 1996). The demonstration of activity against both Gram negative and Gram positive bacteria and fungi is an indication that the plant could be a source of bioactive substances that could have broad spectrum of activity against pathogenic organisms.

The MIC of the extracts on the bacterial isolates ranged from 450 to 1100 µg/ml with the cold extracts demonstrating the lowest values (MIC 600 µg/ml) against *S. aureus*; (MIC 450 µg/ml) against *P. aeruginosa* as seen in Table 5. The MIC values of the ethanol extracts were generally higher and showed that the ethanol extracts were not potent when compared with both cold and hot water extracts of the plants under study. The MIC of the plant extracts against the fungi also ranged from 40 to 85 µg/ml. The MIC of cold extracts (S_{30} and S_{54} *M. alba*) of 50 µg/ml each against *A. niger* were lower than the MIC of the control (streptomycin 60 µg/ml). The MIC of ethanol extracts (70 µg/ml for S_{30}; 75 µg/ml for S_{54}) against both *A. niger* and *A. tamari* were higher than the MIC values of hot extracts (60 µg/ml each for S_{30} and S_{54}) against *A. niger* and (60 µg/ml for S_{30}; 55 µg/ml for S_{54}) against *A. tamari*. Generally, the cold extracts of both S_{30} and S_{54} of *M. alba* showed higher potency indicated by

the lower MIC values. Cold extraction is generally used in folklore medicine, because of its tendency in extracting more of the active ingredients in plants while also preserving the integrity of the bioactives.

Conclusion

Extracts of *M. alba* (S_{30} and S_{54}) could provide alternative to the treatment of both bacterial and fungal infections. The antibacterial and antifungi activities of the plant extracts, probably due to the presence of some phytochemicals and minerals, further consummate its potential in the treatment of some infections. Extraction methods could also play important roles in the folklore medicine potential of the plant extracts as cold extraction possibly protects the integrity of the bioactive substances found in the plant. Future work will focus on isolation, identification and purification of these phytoconstituents and the toxicological evaluation of the plant extracts.

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