PJN
ISSN 1680-5194

PAKISTAN JOURNAL OF NUTRITION

ANSInet
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Pakistan Journal of Nutrition 11 (5): 456-460, 2012
ISSN 1680-5194

Phytochemical Analysis, Nutritional Composition and Antimicrobial Activities of White Mulberry (*Morus alba*)

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Abstract: The phytochemical analysis, nutritional composition and antimicrobial activities of two variants of *Morus alba* were investigated using standard procedures. The leaves were dried under room temperature and then extracted with cold water, hot water and ethanol (99.7% vol./vol.). The extracts were concentrated using rotary evaporator and kept in desiccators for further analysis. The antibacterial and antifungal activities of aqueous (hot and cold) and ethanol extracts of white mulberry plant (*M. alba*) were carried out *in vitro* by agar diffusion-method against some human pathogenic microbes. Antibacterial potential of the *M. alba* (White mulberry plant) extracts were screened against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus faecium*, *Escherichia coli*, *Neisseria gonorrheae*, *Proteus vulgaricus*, while the antifungal properties were done on *Aspergillus niger*, *Aspergillus tamari*, *Fusarium oxysporum* and *Penicilium oxalicum*. Bacitracin and streptomycin were used as the standard reference antibiotics. The phytochemical screening revealed the presence of saponins, phenolics and alkaloids while the antinutrients such as tannins, phytate and oxalate were also present in appreciable amounts. The nutritional evaluation showed that the plant is low in crude protein, crude fat and crude fibre and the mineral compositions confirmed the presence of calcium, zinc, potassium, phosphorus, magnesium. The result also showed that the ethanolic extracts had wider range of activity on the test organisms with high zone of inhibition when compared to the standard antibiotics. The cold extract had lower MIC values compared to the MIC values of the ethanolic extract which indicate that cold extract of the plants are more potent in suppressing microbial growth than the ethanolic extracts. This study supports the folklore use of plants (herbal extracts) in traditional medicines to cure many diseases like diarrhea, intestinal tract, throat, ear infections, fever and skin diseases and its potential in food supplements.

Key words: Phytochemical, nutritional, antibacterial, antifungal, ethanolic extract, organisms

INTRODUCTION

The search for biologically active compounds from natural sources has always been of great interest to scientists looking for new sources of drugs useful in infectious diseases. In recent years a number of studies have been reported, dealing with antimicrobial screening of extracts of medicinal plant between chemical composition, biological and therapeutic activities. The use of such plant is based on pharmacological and taxonomic information of extracts from the leaves, back and roots of such medicinal plants. Their alternative applications for the treatment of other infectious diseases have also been studied. Some of them have been found to have antioxidant, antigenotoxic and insecticidal properties (Tepe *et al*., 2005; Lee and Shibamoto, 2002; Cheng *et al*., 2009).

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 10-20 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. The mature plant contains significant amounts of resveratrol, particularly in stem bark. The fruit and leaves are sold in various forms as nutritional supplements. Unripe fruit and green parts of the plant have a white sap that is intoxicating and mildly hallucinogenic (Cui *et al*., 2008). In traditional and folklore medicine, the fruit is believed to have medicinal properties and is used for making jam, wine and other food products. The fruits contain anthocyanins, a natural food colourants and modulators of mechanisms for various diseases. Due to increasing demand for natural

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food colourants, their significance in the food industry is increasing. Anthocyanins are responsible for the attractive colours of fresh plant foods, producing colours such as orange, red, purple, black and blue. They are water-soluble and easily extractable (Wrolstad, 2001; Hou, 2003).

The white mulberry has a long history of medicinal use in Chinese medicine; recent research has shown improvement in elephantiasis when treated with leaf extract injections and tetanus following oral doses of the sap mixed with sugar (Brown, 1995). The leaves are antibacterial, astringent, diphorectic, odontalgic and ophthalmic (Yeung Him-Che, 1985; Duke and Ayensu, 1985; Brown, 1995). They are taken internally in the treatment of colds, influenza, eye infections and nose-bleed (Yeung Him-Che, 1985; Brown, 1995). An injected extract of the leaves can be used in the treatment of elephantiasis and purulent fistulae (Yeung Him-Che, 1985). The leaves are collected after frosts of autumn and can be fresh but are generally dried (Brown, 1995). The aim of this study was to evaluate the phytochemical constituents and the nutritional values of this plant in order to further utilize it as food supplements. The potentials of the plant extracts on some pathogenic organisms were also investigated.

MATERIALS AND METHODS

The two variants of white mulberry leaves (*M. alba*) were collected from the farm of Forestry Research Institute of Nigeria, Ibadan and identified as S_{14} and S_{34} in the Harberium section of the Institute. The leaves were kept away from sun rays and air dried at room temperature. They were milled to powder using a Maesalab mill (Model 200 LAB) and stored in air tight containers at room temperature until required for further analysis.

Extraction: The powdered fractions of the two variants (S_{14} and S_{34}) of the leaves were transferred into separate closed containers. Ten grams of powdered air-dried plant material was extracted with either 100 ml of cold distilled water, hot distilled water or ethanol (99.7% v/v) in a conical flask, with shaking at 300 rpm for 30 minutes. The process was repeated three times to exhaustively extract the material, followed by storage for 24 h in the refrigerator. After 24 h, each of the extracts was filtered through four layers of gauze, the filter then passed through a Whatman No.1 filter paper. The resulting double filtrates were then concentrated in a rotary evaporator until eventual lyophilization (Eloff, 1998).

Phytochemical screening, nutritional composition and mineral contents: The plant materials were phytochemically screened using the methods of Brain and Turner (1975). 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 the method of Ilelaboye and Pikuda (2009).

Test organisms: The bacterial and fungal isolates 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. All strains were clinical isolates obtained from the Microbiology Laboratory of the Institute of Agricultural Research and Training, Obafemi Awolowo University, Ibadan, Nigeria. Fungal isolates from the same source were used, these being *Aspergillus tamari*, *Aspergillus Niger*, *Fusarium oxysporum* and *Penicillum oxalicum*. All bacterial strains were cultivated in Nutrient Agar Medium (NA) and incubated at 37°C for 24 h while, fungi were cultivated in Potato Dextrose Agar (PDA). This was used for the microbial activity in the disc diffusion assay.

Antimicrobial susceptibility test by disc diffusion assay: Antimicrobial activity of the aqueous and organic extracts of the plant samples were evaluated by the paper disc diffusion method (Brantner and Grein, 1994; Ali *et al*., 1997). For determination of antibacterial activity, bacterial cultures were adjusted to 0.5 McFarland turbidity standards and inoculated onto 15 cm diameter nutrient agar (petri-) plates. For the determination of antimycotic activity, all the fungal isolates were first adjusted to the concentration of 10^6 cfu/ml. All the cultures were inoculated onto Potato Dextrose Agar plates. Sterile filter paper discs diameter (6 mm) impregnated with 100 µl of reconstituted extract (10 mg/ml) to give final concentration of 1mg/disc were placed on the culture plates previously seeded with the 0.5 McFarland and 10^6 cfu/ml cultures of bacteria and fungi respectively. Bacterial cultures were then incubated at 37°C for 24 h, while the fungal cultures were incubated at room temperature (30-35°C) for 48 h. 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 measurement of inhibition zone around each paper disc. For each extract three replicate trials were conducted against each organism.

Determination of Minimum Inhibitory Concentration (MIC): A current definition of the Minimum Inhibitory Concentration (MIC) is the lowest concentration which resulted in maintenance or reduction of inoculums viability (Carson *et al*., 1995). Serial tube dilution technique (Iwaki *et al*., 2006; Khan *et al*., 2007) was used to determine MIC of the extracts against gram-

Table 1: Phytochemical screening of *M. alba*

	Percentage (%)	
Phytochemical constituents	S_{14}	S_{34}
Saponin	4.915	5.005
Phenolics	2.600	2.560
Alkaloid	0.830	0.825
Flavonoids	2.400	2.815

Tests were done triplicates and values expressed as mean in %

Table 2: Phytochemical screening of *M. alba*

	Percentage (%)	
Antinutrient constituents	S_{14}	S_{34}
Tannins	3.455	2.905
Phytate	3.050	2.915
Oxalate	2.905	2.775
Trypsin inhibitor	1.695	1.525

Tests were done triplicates and values expressed as mean in %

Table 3: Nutritional compositions of *M. alba*

	Composition (%)	
Parameters	S_{14}	S_{34}
Crude protein	1.600	1.57
Crude fat	0.045	0.10
Crude fibre	0.605	0.67
Ash content	7.600	6.20
Moisture content	2.650	2.85
Dry matter	97.350	97.15

Tests were done triplicates and values expressed as mean in %

Table 4: Mineral compositions of *M. alba*

	Contents (mg/100 g)	
Parameters	S_{14}	S_{34}
Calcium	1.550	1.510
Phosphorus	6.950	7.650
Zinc	2.400	3.600
Potassium	33.050	31.200
Magnesium	1.495	1.635

Tests were done triplicates and values expressed as mean in %

positive and gram-negative bacteria and fungi. The Minimum Inhibitory Concentration (MIC) of the extracts was determined for each of the test organisms in triplicates. To 0.5 ml of varying concentrations of the extracts, 2 ml of nutrient broth was added (so the extracts were diluted by a factor of 5). Therefore the final concentrations of the extracts were recorded and then a loopful of the test organism previously diluted to 0.5 McFarland turbidity standards for (bacterial isolates) and 10 cfu/ml (for fungal isolates) was introduced to the tubes. The procedure was repeated on the test organisms using the standard antibiotics (bacitracin for bacteria and streptomycin for fungal isolates). A tube containing nutrient broth only was seeded with the test organisms as described above to serve as control. 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 (30-35°C). After incubation the tubes were then examined for microbial growth by observing turbidity.

RESULTS AND DISCUSSION

The present study carried out on the two variants of *M. alba* revealed the presence of bioactive constituents of medicinal values. The phytochemical and antinutrient compounds were analyzed quantitatively and the results were shown in Table 1 and Table 2. The phytochemical analysis of the two variants of *M. alba* showed the presence of all major bioactive compounds with saponin, phenolics and flavonoids having high percentage in both the two variants of *M. alba* screened. The antinutrient compounds were relatively high with tannin being the highest in the two variants. These phytochemicals exhibit diverse pharmacological and biochemical actions when ingested by animals (Amadi *et al*., 2006). Saponin reduces the uptake of certain nutrients including glucose and cholesterol at the gut through intra-luminal physicochemical interactions. Hence, it has been reported to have hypo-cholesterolemic effect (Price *et al*., 1987) and thus may aid in lessening the metabolic burden that would have been placed in the liver. Alkaloids are often toxic to man and may have dramatic physiological activities hence their wide use in medicine (Shelton, 2000). The antinutritional contents include phytate, tannin and Oxalate. Oxalate can complex with most essential trace metals therefore making them unavailable for enzymatic activities and other metabolic activities. Tannins are capable of lowering available protein by antagonistic competition and can therefore elicit protein deficiency syndrome, 'kwashiorkor' Phytic acid has complicated effect in human system including indigestion of food and flatulence (Maynard, 1997). These antinutritional factors can easily be reduced to tolerable limits by proper simple processing techniques such as soaking cooking, frying (Ekpo *et al*., 2004).

The nutritional compositions of S_{14} and S_{34} of *M. alba* revealed low contents of crude protein, crude fat and crude fibre as shown in Table 3. Ash contents for the two variants of *M. alba* were relatively low when compared to ash contents of *Senna siamea* (12.93%), though the ash contents correlate positively with the mineral composition of the plants implying the plants are not good sources of minerals which are necessary for some hormologic functions. Mineral compositions shown in Table 4 reveals that potassium has 33.05 mg/100 g for S_{14} and 31.20 mg/100 g for S_{34}. Generally, the mineral compositions of the plants were low which correlate with the low ash contents of the plants.

The antimicrobial activity of the ethanolic extracts of the *M. alba* variants showed some inhibitory power against the microbes used for this research work with S_{14} extract

Table 5: Antimicrobial activity of ethanolic extracts of *M. alba* (S_{14} and S_{34})

	Zone of inhibition (mm)		
Isolate used	S_{14}	S_{34}	Control*
Staphylococcus aureus	08	08	14
Pseudomonas aureginosa	11	10	17
Streptococcus faecium	10	11	16
Escherichia coli	09	08	21
Neisseria gonorrheae	06	07	24
Proteus vulgaricus	10	08	12
Aspergillus niger	04	05	09
Aspergillus tamari	04	06	14
Fusarium oxysporum	04	04	12
Penicillum oxalicum	05	03	18

*Bacitracin and streptomycin as standard antibiotics

Table 6: Minimum Inhibitory Concentration (MIC) µg/ml of *M. alba* leave extracts

	MIC (µg/ml) (S_{14})			
Organism isolates	Cold H_2O	Hot H_2O	Ethanol	Control*
Staphylococcus aureus	600	650	800	700
Pseudomonas aeruginosa	400	520	900	700
Streptococcus faecium	500	600	1000	850
Escherichia coli	600	670	900	800
Neisseria gonorrheae	500	580	800	800
Proteus vulgaricus	450	570	750	700
Aspergillus niger	50	60	70	60
Aspergillus tamari	40	60	80	60
Fusarium oxysporum	50	65	80	55
Penicilium oxallicum	50	63	75	50
	MIC (µg/ml) (S_{34})			
Organism isolates	Cold H_2O	Hot H_2O	Ethanol	Control*
Staphylococcus aureus	550	650	850	700
Pseudomonas aeruginosa	400	550	900	700
Streptococcus faecium	500	700	1100	800
Escherichia coli	500	750	1000	800
Neisseria gonorrheae	500	650	850	850
Proteus vulgaricus	500	550	750	700
Aspergillus niger	50	65	70	60
Aspergillus tamari	50	60	70	65
Fusarium oxysporum	60	65	85	50
Penicilium oxallicum	60	70	70	55

*Bacitracin and streptomycin as standard antibiotics

showing the highest activity (diameter of inhibition zone 11 mm) against *P. aureginosa* and S_{34} (diameter of inhibition zone 11 mm). The lowest activity was shown by S_{34} (diameter of inhibition zone 03 mm) against *P. oxalicum*. Generally, the results of the plant extracts (Table 5) showed they were effective against most of the microorganisms used. The results of the Minimum Inhibitory Concentration (MIC) of the extracts are shown in Table 6. The results showed that *S. faecium* and *E. coli* have the highest MIC for ethanolic extracts of S_{14} (1000 µg/ml), S_{34} (1100 µg/ml) and S_{14} (900 µg/ml), S_{34} (1000 µg/ml) respectively. MIC of the aqueous extracts were generally lower when compared with MIC of the ethanolic extracts of the plant samples as seen in Table 6. The low MIC values shown by the aqueous extracts of the plants are indication of efficacy and their potential in traditional medicine while the high MIC values obtained for the ethanolic extracts are indication that the extracts are not effective against bacteria and fungi.

Conclusion: From time immemorial, plants have been explored for the use of man as source of food and medicine. These explorations over the years have led to increase in the identification and improvement of plants and plant products for the benefit of mankind. *M. alba* has been identified as a multifunctional versatile plant with enormous economic, nutritional and health potentials. This study revealed the importance and usefulness of the plant. However, future studies are needed to further work on the isolation and characterization of the bioactive ingredients in *M. alba*.

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