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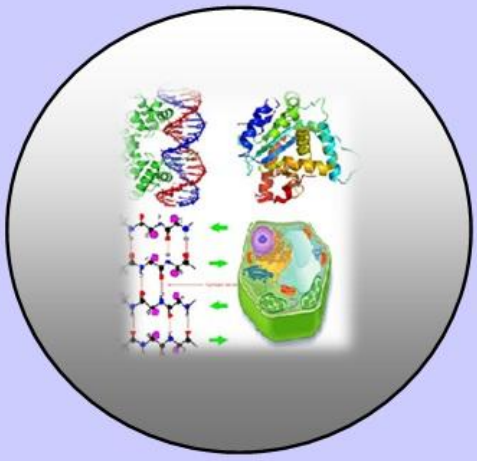

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RESEARCH PAPER
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Inhibition of Microbes by *Lawsonia alba* L. Fruits

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ABSTRACT

Folkloric medicinal application of Lawsonia alba in the treatment of tuberculosis was investigated. Phytochemical analysis revealed the presence of carbohydrates, cardiac glycosides, steroids, triterpenes, alkaloids, tannins and saponins. Hexane (HE), dichloromethane (DCM), ethyl acetate (EA) and methanol (ME) extract of L. alba fruit was evaluated for antibacterial and antifungal activities, against ten pathogenic bacteria and two fungi; Shigella dysenteriae, Salmonella typhi, Corynebacterium ulcerans, Klebsiella pneumonia, staphylococcus aureus, Methicillin resistant staphylococcus aureus (MRSA), Streptococcus pyogens, Baccillus cereus, Escherichia coli and Enterobacter sp, Candida tropicalis, and Candida albicans, using the agar-in-well diffusion method. Determination of zone of inhibition (ZI) showed inhibition ranging from 20-23 mm (HE), 25-30 mm (DCM), 30-33 mm (EA) and 22-25 mm (ME) against the entire test organisms except Methicillin resistant staphylococcus aureus (MRSA), Klebsiella pneumonia, Shigella dysenteriae and Candida albicans. The results of the minimum inhibitory concentration (MIC) showed that EA fraction inhibited the growth of all test organisms at a low concentration of 5 mg/mL. Higher MIC values were observed for DCM (5-10 mg/mL), HE and ME fraction all showed MIC at 10 mg/mL. The microorganisms were completely killed at a higher concentration; EA (MBC/MFC; 10 mg/mL), DCM (MBC/MFC; 10-20 mg/mL), ME and HE (MBC/MFC; 20 mg/mL). Antituberculosis evaluation reveals that the HE extract had the highest activity with MIC of 0.675 mg/mL against Mycobacterium bovis, followed by DCM extract. The results clearly showed that the plant had potential that can be explored in the search for anti-TB drug. This is the first work reported on this plant species.

Keywords: Lawsonia alba; Antituberculosis; Mycobacterium bovis; Antibactrial; Antifungal and Phytochemical screening.

INTRODUCTION

Medicinal plants represent a rich source of antimicrobial agents. Plants are used medicinally in different countries and are a source of many potent and powerful drugs (Chandra, 2013), for centuries, many biological substances have an outstanding role in medicine. They are either used directly or after they have been subjected to certain chemical modification processes. These plants which are medicinal in nature however contain bioactive compounds (Sasidharan et al*.,* 2010) that over the years have been exploited in ayurvedic medicines for the treatment of various ailments. The prevalence of bioactive principles such as tannins, terpenoids, flavonoids, alkaloids, steroids etc. underscores the needs for continuous search for bioactive and active ingredients extracted from plant, though some of the active ingredients of crude extracts become obsolete because of drug resistance (Ojiako, 2014). The prevalence of resistance calls for research in finding new and innovative antimicrobials. Tuberculosis, also called TB, is currently a major health hazard due to multidrug-resistant forms of bacilli (Ramachandran et al., 2014). Global efforts are underway to eradicate TB using new drugs with new modes of action, higher activity, and fewer side effects in combination with vaccines. For this reason, unexplored new sources need be examined, to develop drugs from these new sources. Since ancient times, different plant part extracts have been used as traditional medicines against diseases including tuberculosis. This knowledge may be useful in developing future powerful drugs. Plant natural products are again becoming important in this regard. In an effort to expand the spectrum of anti TB and antibacterial agents from natural resources, *Lawsonia alba* belongs to Anacardiaceae, a family composed of deciduous shrub growing up to 3 metres tall, occasionally becoming a tree of tropical and sub-tropical geographical distribution (Burkil 1985) *Lawsonia alba* has been wide implicated in traditional medicinal application in the treatment of tuberculosis, nausea, fever, cough and generalized body pains, (Burkil 1985, Ruffo et al 2002). In the current investigation carried out, a screening of the methanol, ethyl acetate, dichloromethane and hexane extracts of leaves of *Lawsonia alba* against pathogenic bacteria, fungi and *Mycobacterium bovis* is done in order to detect new sources of antimicrobial and antituberculosis agents.

MATERIALS AND METHODS

Plant materials

The plant material was collected fresh dried powder was used in experimental.

Extraction of plant materials

The pulverized leaves of *Lawsonia alba* (500 g) was carefully weighed and macerated with 95% methanol for one weeks. The extract was decanted, filtered and labeled. The process was repeated three times for exhaustive extraction. The three sets of extracts were combined on confirmation by TLC. The combined methanol extract was partitioned with hexane, dichloromethane and ethylacetate. The extracts were concentrated in vacuum at 40˚C using rotator evaporator and later subjected to air drying to give dried crude extracts.

Phytochemical screening

The hexane, dichloromethane, ethyl acetate and the methanol extracts of the plant was subjected to phytochemical screening using standard techniques (Harborne, 1973). The metabolites tested for included, carbohydrates, tannins, saponnis, flavonoids, anthraquinones, cardiac glycosides, steroids, terpenes and alkaloids.

Antimicrobial studies

The antimicrobial activities of the HE, DCM, EA and ME extracts and standard drugs (Ciprofloxacin, Sparfloxacin and Fluoconazole) were determined using microbial strains and fungi obtained from the Department of Medical Microbiology, Ahmadu Bello University Teaching Hospital, Zaria, Nigeria (ABUTH).The test microorganisms used are *Shigella dysenteriae, Salmonella typhi, Corynebacterium ulcerans, Klebsiella pneumonia, Staphylococcus aureus, Methicillin resistant Staphillococus aureus, Streptococcus pyogens, Baccillus cereus, Escherichia coli Enterobacter sp, Candida tropicalis,* and *Candida albicans*. The well diffusion method of Preeti et al., (2014), was used to determine the antibacterial activity of the test extracts. Pure cultures of the bacterial organisms were inoculated on to Mueller Hinton Agar (MERCK) and incubated for 24 h at 38 ^{o}C. About 5 discrete colonies were aseptically transferred using sterile wire loops into tubes containing sterile normal saline (0.85% NaCl) and were adjusted to a turbidity of 0.5 MacFarland Standard. The suspensions were then inoculated on the surface of sterile Mueller – Hinton Agar plates using sterile cotton swabs. A sterile 6 mm diameter Cork borer was used to make holes (wells) into the set of inoculated Mueller-Hinton Agar. The wells were filled with different concentration of the test extracts. The plates were incubated for 24h at 38 ^{o}C. All the tests were performed in triplicate and the antibacterial activities were determined as mean diameters of inhibition zone (mm) produced by the test compounds.

Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentrations (MIC) were determined for the extracts using micro broth dilution method in accordance with (Vollekova et al., 2001). Serial dilution of the least concentration of the extracts that showed activity were prepared using test tubes containing 9 ml of double strength nutrient broth (OXOID). The test tubes were inoculated with the suspension of the standardized inocula and incubated at 38 ^{o}C for 18 h. Minimum inhibition

Concentrations (MIC) were recorded as the lowest concentrations of the compounds showing no visible growth (turbidity) in the broth.

Minimum Bactericidal Concentration (MBC/MFC)

The minimum bactericidal and minimum fungicidal concentration were determined by aseptically inoculating aliquots of culture, from the minimum inhibition concentration (MIC) tubes that showed no growth, on sterile nutrient Agar plates and incubated at 38^{o}C for bacteria and 34^{o}C for fungi for 48 h. The MBC/MFCs were recorded as the lowest concentration of extracts showing no bacterial growth at all.

Antituberculosis studies

The microbroth dilution method in Sterile 96 microwell plate as described by Oladosu et al., (2013) was employed for the determination of antimycobacterial activity of the extracts. About 100 mg of each extract was transferred into a sterile bottle, dissolved with 0.5 ml dimethylsulphoxide (DMSO) and 0.5 ml distill water. The extracts were further diluted (1:10) in 7H9 Middlebrook broth to give 10 mg/ml concentration. Into each of the 96 microwell plate was transferred 50 µl of sterile 7H9 broth starting from well 2 to 12. To each of the first wells was added 100µl of 10% DMSO in sterile media (prepared by dispensing 0.1 ml of DMSO into 9.9 ml of 7H9 broth as control), 100 µl of 25 µg/ml solution of rifampicin (standard) and 100 µl of each plant extract.

Using a multi-channel pipette, 50µl was carefully removed from well 1 and added to well 2, mixed thoroughly by pipetting up and down four times, and the process continued to well 11 from which 50 µl was withdrawn and discarded.

Organism preparation

500µg of test organism *Mycobacterium bovis*(BCG) freshly thawed stock was inoculated into 50 ml of sterile Middlebrook 7H9/ADC broth medium and incubated at 30^0C for 5-7 days. The optical density of resulting culture was measured using a uv/spectrophotometer. The optical density (OD) of resulting culture determined at 650 nm was approximately 0.2 which is equivalent to 10^9cfu/ml.

Inoculation: The 5-7 day old culture of BCG monitored on UV spectrophotometer at 650 nm (OD 0.2-0.3) was diluted 1/1000 by adding 50 µl cell culture to 50 ml 7H9/ADC medium, where 50 µL of diluted culture was inoculated to all wells of the plate. The plates were incubated at 30 ^{o}C for 7 days and after incubation stained with tetrazolium dye for growth/inhibition of organisms. The column number of the row at which no apparent growth was seen was recorded as activity.

RESULTS AND DISCUSSIONS

Phytochemical screening

Phytochemical screening (Table 1) of the crude methanol, ethyl acetate, dichloromethane and hexane extracts revealed the presence of carbohydrates, cardiac glycosides, alkaloids, tannin, flavonoids, Saponins, steroids and triterpenes. These phytochemicals could be responsible for the antimicrobial and antituberculosis activities exhibited by the extract

Table 1. Phytochemical screening

Metabolites	HE	DCM	EA	ME
Carbohydrate	-	+	+	+
Cardiac glycoside	+	+	+	+
Tannins	-	+	+	+
Saponins	-	-	-	+
Flavonoids	-	+	+	+
Anthraquinones	-	-	-	-
Steroids	+	+	-	+
Triterpenes	+	+	-	+
Glycosides	+	+	+	+
Alkaloids	-	+	+	+

Key: + = present, - = absent, HE = hexane extract, DCM = dichloromethane extracts, EA = Ethyl acetate extracts, ME =Methanol extracts

Antimicrobial screening

The antimicrobial activity of the extract showed that all the extracts exhibited moderate to good antibacterial and antifungal activity against all the pathogens tested *except* except *Methicillin resistant Staphillococus aureus (MRSA), Klebsiella pneumonia, Shigella dysenteriae and Candida albicans* (Table2).

The ethyl acetate extract exhibited the highest zone of inhibition (33 mm) against *Bacillus cereus and Salmonella typhi.* Where as hexane extract exhibited the lowest zone of inhibition (20 mm) against *Corynebacterium ulcerans*, *Salmonella typhi* and *C. tropicalis* (Table3). The ethyl acetate extracts exhibited minimum inhibitory concentration (MIC) 5mg/ml against all the micro organism (Table 4.) The MBC showed that the ethyl acetate extract was bactericidal at 10mg/ml against the entire test microorganism (Table 5.)

The anti-TB evaluation

The Antituberculosis activity of the extracts showed that the hexane and dichloromethane, extract were sensitive against *Mycobacterium* bovis, but ethyl acetate and methanol extracts were not. The hexane extract showed the highest activity with minimum inhibitory concentration of 0.625mg/ml, while dichloromethane extract showed activity with MIC at 1.25mg/ml (Table 6).

Table 2. Sensitivity test of extracts and standard drugs

TEST ORGANISMS	DCM	EA	ME	HEX	CFX	FCZ
Methicillin Rest staph aureus	R	R	R	R	S	R
Staphylococcus aureus	S	S	S	S	S	R
Streptococcus pyogenes	S	S	S	S	S	R
Bacillus cereus	S	S	S	S	S	R
Corynebacteriumulcerans	S	S	S	S	R	R
Salmonella typhi	S	S	S	S	S	R
Shigelladysenteriae	R	R	R	R	S	R
Klepsiellapneumoniae	R	R	R	R	S	R
Enterobactorsp	S	S	S	S	R	R
Escherichia coli	S	S	S	S	S	R
Candida albicans	R	R	R	R	R	S
Candida tropicalis	S	S	S	S	R	S

Key: S= Sensitive,R = Resistance

Table 3. Zones of Inhibition (mm) of the extracts and standard drugs.

test organisms	DCM	EA	ME	HEX	CFX	FCZ
Methicillin Rest staph aureus	0	0	0	0	35	0
Staphylococcus aureus	28	32	24	22	37	0
Streptococcus pyogenes	27	30	24	23	35	0
Bacillus cereus	30	33	25	21	40	0
Corynebacteriumulcerans	25	29	22	20	0	0
Salmonella typhi	29	33	24	20	41	0
Shigelladysenteriae	0	0	0	0	39	0
Klepsiellapneumoniae	0	0	0	0	40	0
Enterobactorsp	26	30	25	21	0	0
Escherichia coli	27	30	24	22	32	0
Candida albicans	0	0	0	0	0	35
Candida tropicalis	28	32	24	20	0	35

Table 4. Result of Minimum Inhibitory Concentration (MIC).

Test Organisms	DCM	EA	ME	HE
Staphylococcus aureus	5.0	5.0	10.0	10.0
Streptococcus pygenes	5.0	5.0	10.0	10.0
Bacillus cereus	5.0	5.0	10.0	10.0
Corynebacterimulcerans	10.0	5.0	10.0	10.0
Salmonella typhi	5.0	5.0	10.0	10.0
Enterobactersp	10.0	5.0	10.0	10.0
Escherichia coli	5.0	5.0	10.0	10.0
Candida tropicalis	5.0	5.0	10.0	10.0

Key: DCM – Dichloromethane, EA – Ethyl acetate, ME – Methanol, HE – Hexane

Table 5. Minimum bactericidal/fungicidal concentration (MBC/MFC) of the extracts in (mg/ml)

Test Organisms	DCM	EA	ME	HE
Staphylococcus aureus	10	10	20.0	20.0
Streptococcus pygenes	10.0	10.0	20.0	20.0
Bacillus cereus	10.0	10.0	20.0	20.0
Corynebacterimulcerans	20.0	10.0	20.0	20.0
Salmonella typhi	10.0	10.0	20.0	20.0
Enterobactersp	20.0	10.0	20.0	20.0
Escherichia coli	10.0	10.0	20.0	20.0
Candida tropicalis	10.0	10.0	20.0	20.0

Key: DCM = Dichloromethane, EA = Ethylacetate, ME =Methanol, HE =Hexane

Table 6. Minimum Inhibitory Concentration (MIC) of the extracts against *Mycobacterium bovis*

Extract Concentration (mg/ml)	**Hex**	**Dcm**	**EA**	**ME**	**Rifampicin**
5	NA	**+**	**+**	NA	**+**
2.5	NA	**+**	**+**	NA	**+**
1.25	NA	**+**	**+***	NA	**+**
0.675	NA	**+***	NA	NA	**+**
0.3125	NA	NA	NA	NA	**+**

Key:-= no inhibition; **+** = inhibition; **+*** = MIC

Recently there has been considerable interest in the use of plant material as an alternative method to control pathogenic microorganism (Aqil et. al., 2005) and many components of plants products have been shown to be specially targeted against resistant pathogenic bacteria (Nostro et. al., 2006).

The emergence of multidrug resistant strain of many pathogens is a serious threat and makes chemotherapy more difficult. Moreover, the current cost of most of the chemotherapeutic agents is unbearable to the public especially in developing countries (Chandra, 2013).Therefore attempts must be made towards the development of effective natural, non-toxic drug for treatment. The present work was done to explore the antimicrobial and antituberculosis property of *Lawsonia alba*.

Phytochemical analysis carried out on the plant extracts revealed the presence of constituents which are known to demonstrate medicinal as well as physiological activities (Jain and Bari 2010). Phytochemical screening of the plant extracts revealed the presence of phytochemicals such as carbohydrates, tannins, saponins, cardiac glycosides, steroid, triterpenes and alkaloids. These could be responsible for high antimicrobial activity and antituberculosis activity demonstrated bythe plant extracts. Tannins, saponins and alkaloids have been reported to have pronounced physiological effect particularly on the nervous system (Simkin et al 2008).Tannins encompass a heterogeneous group of compounds and polymers (polyphenols). In general their non-specific activity has been ascribe to their ability to complex metal ions, scavenge radicals and reduce active oxygen species and form tight complexes with a wide array of proteins and polysaccharides (Haslam 1996). Hence, they have antioxidative properties. Saponins are known to produce inhibitory effect on inflammation (Just et al 1998) Steroid and Triterpenes have been reported to have antibacterial properties (Raquel 2007).

Alkaloids have been reported for their cytotoxic, analgesic, antisplasmodic and antibacterial (Okwu 2004) properties. Glycosides are known to lower the blood pressure according to many reports (Nyarko and Addy). The presence of these phytochemicals in *Lawsonia alba* extracts validates the claim by the traditional healers in the treatment of several ailments. The antimicrobial sensitivity test of the leave extracts of *Lawsonia alba* showed that the extracts have moderate to good activity. The Determination of zone of inhibition (ZI) showed inhibition ranging from 20-23 mm (HE), 25-30 mm (DCM), 30-33 mm (EA) and 22-25 mm (ME) against the entire test organisms except *Methicillin resistant Staphillococus aureus (MRSA), Klebsiella pneumonia, Shigella dysenteriae and Candida albicans.* The ethyl acetate extract had the highest zone of inhibition of 33mm against Bacillus cereus and salmonella typhi. The results of the minimum inhibitory concentration (MIC) showed that EA fraction inhibited the growth of all test organisms at a low concentration of 5 mg/mL. Higher MIC values were observed for DCM (5-10 mg/mL), HE and ME fraction all showed MIC at 10 mg/mL. The microorganisms were completely killed at a higher concentration; EA (MBC/MFC; 10 mg/mL), DCM (MBC/MFC; 10-20 mg/mL), ME and HE (MBC/MFC; 20 mg/mL). Antituberculosis evaluation reveals that the hexane extract had the highest activity with MIC of 0.675 mg/mL against *Mycobacterium bovis*, the dichloromethane extract was also active at MIC of 1.25mg/ml while other extracts were not active. The results clearly showed that the plant had potential that can be explored in the search for anti-TB drug.

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REFERENCES

Aqil F, Khan M.S., Owais M., and Ahmad I.. Effects of certain bioactive plant extracts on clinical isolates of beta-lactamase producing *methicillin- resistant Staphylococcus aureus.* Journal of Basic Microbiology. 45 (2005), pp.106-114.

Burkil. H. M.(1985 - 2004) The Useful Plants of West Tropical Africa.Publisher Royal Botanic Gardens; Kew. http://www.aluka.org/

Chandra, M. Antimicrobial Activity of Medicinal Plants against Human Pathogenic Bacteria International Journal of Biotechnology and Bioengineering Research, 4(7) (2013), pp. 653-658.

Harborne JB; Phytochemical methods: A guide to modern techniques of plant analysis. 2nd edition, *Chapmann and Hall*, London, 1973; 279 pp.

Haslam E; Natural polyphenols (Vegetable tannins) as Drugs: possible modes of action. Journal of Natural Products 1996: 59:205-215.

Jain PS, Bari SB; Isolation of Lupeol, Stigmasterol and Campesterol from Petroleum Ether Extract of Woody Stem-Bark of *Wightiatinctoria*. Asian Journal of Plant Sciences, 2010: 9 (3): 163- 167.

Just M .J, Recio M.C, Giner RM, Cueller MU, Manez S, Billia AR, Rios JL; Anti inflammatory activity of unusual lupine saponins from *Bupleurumfruticescens*, 1998: 64: 404-407.

Nostro A, Cellini L. and DiBartolomeo S. Effect s of combining extracts (from propolis or *Zingiberofficinale*) with clarithromycin on Helicobacter pylori. Phytotherapy Research. 20 (3) (2006), pp. 187-190.

Nyarko AA, Addy ME; Effects of aqueous extract of Adeniacissampeloides on blood pressure and serum analyte of hypertensive patients. Phytotherapy Res., 1990: 4(1): 25-28.

Ojiako EN; Phytochemical analysis andantimicrobials screening Of *Moringa Oleifera* Leaves Extract. International Journal of Engineering and science, 2014; 3 : 32-35

Okwu DE; Evaluation of the chemical composition of indigenous spices and flavoring agents. Global. Journal of Pure and applied sciences, 2001: 7(3):455-459.

Okwu DE; Phytochemicals and vitamin content of indigenous species of southeastern Nigeria. Journal of Sustainable Agriculture and Environment, 2004:6(1): 30-37.

OladosuPO,Isu NR, Ibrahim K, Orishade AT, Oladepo D , Lovett L; Antituberculosis activity of bioactive compounds from fruits extracts of Acacia nilotica. Journal of microbiology Research, 2013; 3 : 247-254.

Preeti G, Uday V, Singh T; Phytochemical screening and antimicrobial activity of some medicinal plants against oral flora. Asian Pac. Journal of Health Sci. , 2014 ;1 : 255-263.

Ramachandran S. S., Balasubramanian S. Plants: A Source for New Antimycobacterial Drugs Planta Med, 80 (2014), pp. 9–21

Raquel FE; Bacterial lipid composition and antimicrobial efficacy of cationic steroid compounds. Biochemicaet Biophysica Acta. 2007: 2500-2509.

Ruffo, C.K; Birnie, A. ;Tengnas, B. Edible Wild Plants of Tanzania Publisher Regional Land Management Unit; Nairobi. 2002

Sasidharan S., Chen Y, Saravanan D, Sundram KM, Yoga L L; Extraction, isolation and characterization of bioactive compounds from plants extracts. African Journal of Traditional, Complementary and Alternative Medicine.2010; 8) :1-10.

Simkin AJ, Moreau H, Kuntz M, Pagny G, Lin C, Tanksley S, Mccarthy J; An investigation of carotenoid biosynthesis in coffeacanephora and coffea Arabica. Journal of plant physiology, 2008: 165, 1087-1106.

Vollekova A , Kostalova D, SochorovaR;Isoquinoline alkaloids from *Mahoniaaquifolium* stem bark are active against Melsseziaspecies.Folia Microbiology, 2001; 46 : 107-111.

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