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## Phytochemical Screening of Leaves from *Mangifera indica* and *Morus alba* for Bioactive Compounds

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### ABSTRACT

The aqueous, methanol and DMSO extracts from leaves of *Mangifera indica* and *Morus alba* were screened for bioactive compounds. Antimicrobial activity and MIC (mg/ml) were determined using agar-well diffusion and serial dilution methods respectively against human pathogenic bacteria and fungi. Antioxidant activities (%) were determined against DPPH free radicals, superoxide anion and Fe<sup>2+</sup> whereas antiurease activity (%) was by indophenol method. The time taken to set paralysis and death of *Pheritima posthuma* was observed after released into extract. Phytochemical analysis detected carbohydrates, alkaloids, phenols, flavonoids, terpenoids, saponins, tannins, steroids, resins, anthraquinones, cardiac glycosides and phlobatannins. All extracts more or less showed the activity against Gram-positive and -negative bacteria producing zone of inhibition 15±0.57 to 43.66±0.66 mm and MIC values 13±1.64 to 100±0.35 mg/ml. Similarly, for fungi, zone of inhibition and MIC values were 9±0.57 to 34±0.57 mm and 23±1.25 to 115±0.55 g/ml respectively. Activities against DPPH, superoxide anion and Fe<sup>2+</sup> free radicals were found 13.68±0.74 to 93.70±0.76 %, 50.23±0.43 to 95.7±0.21 % and 52.76±0.23 to 98.82±0.42% respectively, of these, the maximum activities surpassed the n-propyl gallate (91.31 %) and EDTA (98.05 %). Similarly, urease was also inhibited (33 to 95.23%) by all extracts. All extracts were also caused paralysis followed by subsequent death (13.5/26.87 to 39/60.06 min) of *P. posthuma*, of these methanolic extract from *M. indica* was least than that by albendazole (30.45/52 min) and piperazine citrate (14.35/36 min). The results provide scientific basis for traditional use of these extracts as affordable strategies for treatments of infectious, inflammatory and degenerative diseases, caused by bacteria, fungi, free radicals, urease and helminths.

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### INTRODUCTION

The use of medicinal plants as herbal remedies for the treatment of diseases and maintenance of health is an effective ancient practice since civilization on earth. Interestingly, they are still widely used for same purposes all over the world especially individuals and communities from rural areas of developing and third

world countries who have difficulty in gaining access to modern medicines use medicinal herbs and plants, as an alternative to conventional treatment for their recovery (Doughari and Manzara 2008; Muanda *et al.*, 2011). According to World health organization (1995) about 80 % of the world population is taking interest in indigenous medicinal plant remedies. The development

and prevalence of multidrug resistance microbial pathogens, and the recent appearance of strains with reduced susceptibility to currently being used antibiotics are increasing at an alarming rate worldwide. Another big concern is that the drug-resistant bacteria and fungal pathogens have further complicated the treatment of infectious diseases especially in AIDS and cancer patients (Rajendran and Ramakrishnan 2009). Besides this, the drug development process is very costly, pharmaceutical companies often concentrate on finding antimicrobials similar to the ones already found to reduce the risk of producing an unmarketable drug. This means that it is easy for microorganisms to develop resistance to a similar drug to which it already has resistance (Cunha 1998). Therefore, there is a continuous and urgent need to search for new infection-fighting strategies (Sieradzki 1999). Free radicals contribute to more than one hundred disorders in humans including atherosclerosis, arthritis, ischemia and reperfusion injury of many tissues, central nervous system injury, gastritis, cancer, and AIDS. Free radicals due to environmental pollutants, radiation, chemicals, toxins, deep-fried and spicy foods as well as physical stress, cause depletion of immune system antioxidants, change in gene expression, and induce abnormal proteins. The oxidation process is one of the most important routes for producing free radicals in food, drugs and even living systems. Currently available synthetic antioxidants like butylated hydroxyanisole, butylated hydroxy toluene, tertiary butylated hydroquinone and gallic acid esters, have been suspected to cause or prompt negative health effects. Hence, strong restrictions have been placed on their application and there is a trend to substitute them with naturally occurring antioxidants (Pourmorad *et al.*, 2006; Mathur *et al.*, 2010). Urease the most prominent protein component of several microorganisms (*Helicobacter pylori*, *Proteus mirabilis*, *P. vulgaris*, and *Ureaplasma urealyticum*), is involved in hydrolysis of urea into ammonia which neutralizes acids of stomach, thus infections of gastrointestinal and urinary tract. The formation of struvite stones in urinary tract is associated with urea-splitting *U. urealyticum*, which may lead to the chronic inflammation of kidney and its pelvis. Moreover, urinary catheter obstruction in patients causes colonization of urease-producing microorganisms, essentially *P. mirabilis*. Overproduction of ammonia by infectious

microorganisms may contribute to ammonia encephalopathy or hepatic coma. Furthermore, high concentration of ammonia disturbs mucosal permeability, in particular, hydrogen ions passage through mucosal surface and causes the formation of peptic ulcers (Estiu and Mer 2004; Khan *et al.*, 2010). *H. pylori* whole cell can stimulate an oxidative burst in human neutrophils which leads to formation of the highly toxic product monochloramine (Tahseen *et al.*, 2010). Parasitic worms are also challenging pathogens for human beings, they pose a major threat to public health and contribute to prevalence of malnutrition, anaemia, eosinophilia, and pneumonia (Kulkarni *et al.*, 2010). Unfortunately, currently being used anthelmintic drugs have adverse effects and their indiscriminate use is leading to resistance of parasites (Devi *et al.*, 2009), therefore, there is a foremost problem in treatment of helminths diseases. Hence there is an increasing demand towards natural anthelmintics. Therefore, it is important to search for novel therapeutic agents, other than synthetic, that are both highly effective and safe.

On the other hands, plants are known to provide a rich source of anthelmintics, antibacterial, antifungal, urease inhibitors and antioxidants. Though much work has been done on ethno-medicinal plants, but there is still needed to seek plants with medicinal value to combat diseases. Because medicines from plants are considered as time tested, effective, cheap, easily available, affordable, and safe for both human use and environment friendly (Iwu *et al.*, 1999; Fazly-Bazzaz *et al.*, 2005). In contrast to current combination therapies, however, plant-based drugs contain a mixture of multiple components thereby saving considerable time and expense (Karnath 2002). The WHO (1995) has supported and recommended the use of traditional medicine provided they are proven to be efficacious and safe. Therefore, the present study was undertaken to screen the bioactive compounds in leaves of some indigenous fruiting plants which are untapped sources of medicines and to evaluate them for antibacterial, antifungal, antioxidant, urease inhibition and anthelmintic activities.

## METHODS AND MATERIAL

### Collection and preparation of plant material

Fresh, healthy, and mature leaves of *Mangifera indica* and *Morus alba* were collected from Garden of the Sindh Horticulture Research Institute, Mirpurkhas. The leaf samples were collected in sterile polyethylene bags.

Leaves were washed once with tap water, rinsed with distilled water, and air-dried at room temperature. Air-dried leaves were chopped into small pieces and ground into fine powder in liquid nitrogen using porcelain mortars and pestles.

#### Extraction procedure

Powder of air-dried leaves (100 g) was transferred in 1000 ml blue cap Schott Duran (GL 45) bottles containing 500 ml water, methanol, and dimethyl sulfoxide (DMSO), and samples were completely submerged in the solvents. The capped bottles were kept in a shaking water bath at 50 °C for 48 hrs. After that, the extracts were filtered using Whatman filter paper no. 2 (125mm) and each filtrate was centrifuged at 10000 rpm for 10 minutes. Supernatants were collected in glass beakers, concentrated under reduced pressure at 45 °C and freeze-dried. The extracts were stored in universal sampling vials of known weight under codes aqueous extract (AE), DMSO extract (DE) and methanol extract (ME) at 4 °C until use. The solvents were removed under the vacuum at temperature below 50 °C and the extracts were freeze-dried. For activity assay, the required quantity of each extract was dissolved in DMSO (Khumpook *et al.*, 2018).

#### Phytochemical screening

Phytochemical screening of each extract for presence and absence of carbohydrates, alkaloids, phenols, flavonoids, terpenoids, saponins, tannins, steroid, resins, anthraquinones, cardiac glycosides and phlobatannins using standard procedures as described by Sofowora *et al.*, (1993) and Radhika *et al.*, (2008).

#### Test Microbial Pathogens

Clinical strains of Gram-positive (*Bacillus subtilis* (Gu *et al.*, 2019), *B. cereus*, *Staphylococcus aureus*, *S. pyogenes* and *S. pneumoniae*), Gram-negative (*Escherichia coli*, *H. pylori*, *Pseudomonas aeruginosa*, *Shigella flexneri*, *Klebsiella pneumoniae* and *Salmonella typhimurium*) bacteria and fungi (*Trichophyton longifusus*, *Candida albicans*, *Aspergillus flavus*, *Microsporum canis*, *Fusarium solani* (Daba *et al.*, 2016) and *Candida glabrata*) acquired from Plant Bioscreening Laboratory of HEJ Research Institute of Chemistry, University of Karachi, Pakistan. Prior to test, each bacterial strain was sub-cultured on nutrient agar medium and incubated at 37 °C for 24hrs. Fungal strains were sub-cultured on sabouraud dextrose agar (SDA) medium and incubated at 28 °C for 15 days.

The freshly revived test microbes were preserved at 4 °C in aliquots.

#### Assay for Antimicrobial Activity

The antimicrobial activity of each plant extract was determined using agar-well diffusion method (Coolborn and Bolatito 2010; Duru and Oyedineke 2010). With a sterile cork borer, wells of equal depth ( $\Delta=5\text{mm}$  diameter) were dug on solidified nutrient agar and SDA media plates respectively for test bacterial and fungal pathogens. 0.2 ml of the log phase culture of test bacterial strains was seeded on the surface of nutrient agar medium while that of fungal strains was seeded on SDA medium. The wells were aseptically filled up separately with the extracts (0, 50, 10, 20, 30, 50 and 100 mg/ml) avoiding splash and overfilling. Imipenem (thienamycin-formamidine) (15  $\mu\text{g/ml}$ ) was used as broad-spectrum antibacterial standard whereas miconazol (150  $\mu\text{g/ml}$ ) and amphoteroicin B (30  $\mu\text{g/ml}$ ) were used as broad spectrum antifungal standard drugs. For every experiment, a sterility check (5% v/v aqueous DMSO and medium), negative control (5% v/v aqueous DMSO, medium and inoculum) and positive control (5% aqueous DMSO, medium, inoculum and standard drug) were included. The positive antibacterial and antifungal activities were established by the measurable zones of inhibition after incubation at 37 °C for 48 hrs and 15 days for bacteria and fungi respectively.

#### Minimum Inhibitory Concentration

The extracts that showed antimicrobial activity were later tested to determine the minimal inhibitory concentration (MIC) for each bacterial and fungal strain. MIC was determined according to the method of Sahn, and Washington (1990) and Radhika *et al.*, (2008). Two hundred milli gram extract was dissolved in 1mL of nutrient broth and subsequently transferred. One millilitre from the first test tube to the next test tube and this continues up to the seventh test tube. Then 20  $\mu\text{l}$  fresh cultures of bacterial and fungal test strains were inoculated into each test tube, mixed thoroughly, and incubated at 37°C for 48hrs and 15 days respectively. The inoculum from tubes that showed no growth was sub-cultured onto extract free agar plates, incubated for another 48 hrs at 37°C and 15 days respectively for bacteria and fungi. The lowest concentration of each extract in each treatment, showing no visible growth after incubation under conditions as mentioned above were recorded as MIC values.

## Determination of antioxidant activities

### DPPH radical scavenging assay

Test samples were allowed to react with stable free radical, 1, 1-Diphenyl-2-picrylhydrazyl radical (DPPH) (from Sigma Aldrich) for half an hour at 37 °C. The concentration of DPPH was kept as 300 µM. The test samples (500 µg/ml) were dissolved in DMSO while the DPPH solution was prepared in ethanol. Propyl gallate was used as antioxidant standard (1 mM/ml). After incubation, decrease in absorption was measured at 515 nm using multiplate reader (Spectra MAX-340). Percent radical scavenging activity (% RSA) by samples was determined in comparison with a DMSO treated control group. Percentage of RSA was calculated in comparison with control by using the following formula:

$$\% \text{ RSA} = 100 - \left\{ \left( \frac{\text{OD test extract}}{\text{OD control}} \right) \times 100 \right\}$$

### Superoxide anion scavenging assay

Measurement of superoxide radical scavenging activity was carried out by the modified method used by Ferda (2003). The reaction mixture comprised of 40 ml of 280 mM β-nicotinamide adenine dinucleotide reduced form (NADH), 40 mL of 80 M nitro blue tetrazolium (NBT), 20mL phenazine methosulphate (PMS) 10 mL of 1 mM sample and 90 mL of 0.1 M phosphate buffer (pH 7.4). Reagents were prepared in buffer solution and the sample in DMSO. The reaction was performed in a 96-well microtiter plate at room temperature and absorbance was measured at 560 nm. The formation of superoxide was monitored by measuring the formation of water-soluble blue formazan dye. A lower absorbance of reaction mixture indicates a higher scavenging activity of the sample. The percentage of RSA was determined in comparison with control by using formula as mentioned above in DPPH assay.

### Metal chelation assay

The Fe<sup>2+</sup>-chelating activity was determined according to the modified method of Kexue *et al.*, (2006). The Fe<sup>2+</sup> was monitored by measuring the formation of ferrous ion-ferrozine complex. The extract (500 µg/ml) was mixed with 0.0625 mM FeSO<sub>4</sub> and 0.5 mM ferrozine then mixture was shaken, and left at room temperature for 10 min. The absorbance of resulting mixture was measured at 562 nm. A lower absorbance of reaction mixture indicated a higher Fe<sup>2+</sup>-chelating ability. EDTA (1mM) was used as Fe<sup>2+</sup>-chelating standard. The percentage of

Fe<sup>2+</sup>-chelating activity was calculated in comparison with a control by using the formula as mentioned above in DPPH assay.

### Urease inhibition assay

Urease activity was determined by measuring ammonia production using the indophenol method as described by Weatherburn (1967). Reaction mixtures (200 µl) at pH 6.8 comprising 25 µl of urease (jack bean) solution and 55 µl of buffers containing 100 mM urea were incubated with 5µl of the test extract (500µg/ml) at 30 °C for 15 min in 96-well plates. 45 µl phenol reagent (1 % w/v phenol and 0.005 % w/v sodium nitroprusside) and 70 µl of alkali reagent (0.5 % w/v NaOH and 0.1 % active chloride NaOCl) were added to each well. Thiourea was used as the standard inhibitor of urease. The increasing absorbance was measured at 630 nm after 50 min, using a microplate reader as mentioned above. The results from five observations were processed by using softMax Pro software (Molecular Device, USA). Percentage inhibitions were calculated from the formula as mentioned above in DPPH assay.

### Anthelmintic activity

The anthelmintic assay was carried according to the method of Ajaiyeoba *et al.*, (2001). Five adult earthworms (*Pheritima posthuma*) were released into 50 ml solutions of three different concentrations (20, 50 and 100 mg/ml each) of control drugs (Piperazine citrate, Albendazole), extracts from leaves and distilled water. Observations were made for the time taken to set paralysis (P) and death (D) of individual worms. Time for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Death was concluded when the worms neither moved when shaken vigorously nor when dipped in warm (60 °C) followed with fading away of their body colors.

### Statistical analysis

Antimicrobial and antioxidant activities of each extract were expressed as mean ± standard error of mean (SEM) of five observations. Data of each extract were separately analyzed using the one-way analysis of variance followed by the student-Waller Duncan test at P<0.05 significance level. A statistical package (CoHort version-6) was used for the data analysis.

## RESULTS

### Phytochemical screening

Phytochemical analysis qualitatively investigated that extracts from leaves of two indigenous fruiting plants *M. indica* and *M. alba* were found to contain biologically active compounds which have been reported to serve as potential plant drugs in herbal medicine. Among the tested leaf extracts, in that of *M. indica*, steroid, anthraquinones and cardiac glycosides, and in *M. alba* phlobatannin and terpenoids were not detected (Table

1). Further, AEs, DEs and MEs from *M. indica* and *M. alba* were found to contain only 5 and 6, 9 and 10, and 8 to 9 tested phytochemical groups, respectively. These results substantiated the several reports on phytochemical analysis of plant leaves that plants contain various extractable bioactive secondary metabolite compounds such as phenols, terpenoids, tannins, alkaloids, flavonoids, steroids, and glycosides etc in appreciable quantities for defense and physiological activities (De and James 2002; Mallikharjuna *et al.*, 2007).

Table 1. Phytochemicals in leaves of *Mangifera indica* and *Morus alba*.

Phytochemicals	<i>Mangifera indica</i>			<i>Morus alba</i>		
	AE	DE	ME	AE	DE	ME
Carbohydrates	+	+	-	+	+	-
Alkaloids	-	+	+	+	+	+
Phenols	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+
Terpenoids	-	+	+	-	-	-
Saponins	-	+	+	-	+	+
Tannins	+	+	+	-	+	+
Steroids	-	-	-	-	+	+
Resins	+	+	+	+	+	+
Anthraquinones	-	-	-	+	+	+
Cardiac glycosides	-	-	-	-	+	+
Phlobatannins	-	+	+	-	-	-

AE aqueous extract, DE DMSO extract, ME methanol extract, + present, - absent.

### In-vitro Biological activities

Based on the presence of bioactive phytochemical groups all leaf extracts were tested for *in-vitro* biological activities such as antimicrobial, antioxidant, urease inhibition and anthelmintic.

### Antibacterial activity

When the efficacy of these extract were tested *in-vitro* against eleven strains of human pathogenic bacteria, all extract were found to exhibit statistically significant ( $P < 0.05$ ) activity on all the test Gram-positive and -negative bacteria producing zone of inhibition ranging from  $15 \pm 0.57$  to  $43.66 \pm 0.66$  mm against *S. typhimurium* and *P. aeruginosa* by AE from *M. indica* and DE from *M. alba* respectively except AE from *M. indica* which could not inhibit the growth of *B. subtilis*, *B. cereus*, *H. pylori*, *K. pneumoniae* and *S. flexenari* (Table 2). Among extracts, AE and DE from *M. indica* significantly inhibited the *S. aureus* whereas ME was significant against *E. coli*. Similarly, among *M. alba* extracts, the antibacterial activity of AE

and ME was significant against *S. typhimurium* and *P. aeruginosa* respectively and that of DE was significant against *B. cereus* and *P. aeruginosa* (Table 2). AE from *M. alba* and *M. indica* showed the minimum inhibition activity against *B. subtilis* and *S. typhimurium* respectively. Similarly, DE and ME from both *M. indica* and *M. alba* showed the least activity against *K. pneumoniae* and *S. flexenari* respectively (Table 2). The spectra of antibacterial activities displayed by these extracts were explained by the presence of biologically potent antibacterial phytochemicals such as flavonoids, tannins, saponins steroids, phenolic acids, anthocyanins, alkaloids and terpenoids in leaves of these two indigenous fruiting plants (Hu 2000; Vaghasya *et al.*, 2011). The activity of extracts against the Gram-negative (*E. coli*, *H. pylori*, *P. aeruginosa*, *S. flexenari*, *K. pneumoniae* and *S. typhimurium*) is impressive because Gram-negative bacteria tend to have higher intrinsic resistance to most antimicrobial agents (Ndukwe *et al.*, 2005). On the other hand, activity against Gram-positive bacteria especially

cocci are interesting as they still predominate as a cause of nosocomial- and community-acquired infections. Additionally, these bacteria are able to acquire resistance to frequently used drugs rapidly through selective pressure of the environment and via the genetic evolution of bacteria (eljaszewicz *et al.*, 2000).

### Antifungal activity

Extracts from leaves of both selected fruiting plants also showed the statically significant ( $P < 0.05$ ) activity against all six tested human pathogenic fungal strains producing zone of inhibition ranging from  $9 \pm 0.57$  to  $34 \pm 0.57$  mm against *C. glabberata* and *F. solani* by AE and DE from *M. alba* respectively except AE from *M. indica* which could not inhibit the *M. canis* whereas AE and DE from *M. alba*

did not the *M. canis* and *F. solani*, and *A. flavus* respectively. Within the various tested extracts, DE and ME from both plants the maximum activity against *F. solani*. Similarly, AE from both plants was found to have maximum activity against *A. flavus*, (Table 2). The minimum activity showed by DE from *M. indica* was against *T. longifusum* whereas that from *M. alba* was against *C. albicans*. The minimum fungal inhibition by AE and ME from both selected plants was against *C. glabberata* (Table 2). These results showed correlation with those of several reports on synthesis of antifungal substances by plants in response to microbial attack and it should not be surprising that they have been found to be still effective against a wide array of pathogenic microorganisms, when tested *in-vitro* (Idris *et al.*, 2009; Kanwal *et al.*, 2010; Tiwari *et al.*, 2011a).

Table 2. Antimicrobial activity of extracts from leaves of *Mangifera indica* and *Morus alba*.

Microbial pathogens	Zone of inhibition (mm)					
	<i>Mangifera indica</i>			<i>Morus alba</i>		
	AE	DE	ME	AE	DE	ME
<i>Bacillus subtilis</i>	NI	25.00±0.57	23.33±0.88	20.66±0.88	37.00±0.57	29.00±0.57
<i>B. cereus</i>	NI	31.33±1.20	21.00±1.00	24.00±1.00	42.33±0.33	31.66±0.66
<i>Escherichia coli</i>	19.66±1.20	22.66±0.33	37.33±0.33	25.66±0.66	36.00±0.57	26.00±0.57
<i>Helicobacter pylori</i>	NI	29.00±0.57	23.34±0.57	32.00±0.88	35.00±0.57	33.00±1.2
<i>Klebsiella pneumoniae</i>	NI	20.66±0.88	33.33±0.88	24.66±1.33	29.33±0.88	34.00±0.57
<i>Pseudomonas aeruginosa</i>	17.33±0.66	21.33±1.66	30.00±0.57	32.66±0.88	43.66±0.66	36.66±0.88
<i>Salmonella typhimurium</i>	15.00±0.57	29.33±0.88	28.66±1.20	43.00±0.57	35.66±0.88	32.00±1.15
<i>Shigella flexenari</i>	NI	33.33±1.20	20.66±1.20	32.66±0.66	35.33±0.88	24.33±0.88
<i>Staphylococcus aureus</i>	24.00±1.15	35.33±0.33	30.66±0.66	17.66±0.66	36.33±0.88	31.00±0.57
<i>S. pyogenes</i>	21.66±0.66	31.33±0.66	33.33±1.20	25.00±1.00	33.66±0.66	26.66±1.20
<i>S. pneumoniae</i>	21.00±1.52	30.00±0.57	29.00±0.57	36.33±0.33	35.00±0.57	25.00±0.57
<i>Trichophyton longifusum</i>	14.66±0.66	15.00±0.57	19.00±0.57	14.33±0.88	23.00±0.57	29.66±0.33
<i>Candida albicans</i>	12.00±0.57	14.33±0.88	17.33±0.33	11.00±0.57	17.00±0.57	18.66±0.88
<i>Aspergillus flavus</i>	21.33±0.33	18.33±0.33	21.33±0.33	17.66±0.33	20.66±0.33	25.33±0.88
<i>Microsporum canis</i>	NI	15.66±0.88	18.33±0.33	NI	NI	14.66±0.33
<i>Fusarium solani</i>	13.66±0.66	21.00±0.57	23.00±0.57	NI	34.00±0.57	30.00±1.00
<i>Candida glabberata</i>	10.33±0.66	17.33±0.33	14.00±0.57	9.00±0.57	25.00±0.57	12.33±1.33

NI: no inhibition.

### Minimum inhibitory concentration

The results of MIC showed that the least concentrations of all tested potent extracts required for inhibition of test bacterial pathogens were in range of  $13 \pm 1.64$  (ME from *M. alba* against *S. aureus*) to  $100 \pm 0.35$  mg/ml (AE from *M. alba* against *S. flexenari*) mg/ml. However, among all potent extracts ME from *M. indica*, DE and ME from *M. alba* had the lowest MIC value against six (*E. coli*, *K. pneumoniae*, *S. typhimurium*, *S. flexenari*, *S. pyogenes* and

*S. aureus*), two (*S. pneumoniae* and *B. cereus*) and (*P. aeruginosa*, *H. pylori* and *B. subtilis*) test bacterial pathogens respectively, thus indicating a high potency (Table 3). Similarly, the MIC values against test fungal pathogens were found in range of  $23 \pm 1.25$  (DE from *M. indica* against *A. flavus*) to  $115 \pm 0.55$  mg/ml (AE from *M. indica* against *C. glabberata*) mg/ml. Among all fungicidal extracts, only DE and ME from *M. indica* and ME from *M. alba* demonstrated the least MIC values against one (*A.*

*flavus*) and two (*F. solani* and *C. glabberata*), and three (*T. longifusus*, *C. albicans* and *M. canis*) respectively, thus indicating a high potency among tested crude extracts (Table 3). Although the antimicrobial activities of these tested extracts were found to be less as compared to standard antimicrobials, may be due to the fact that it was crude and required further purification. However, still can play significant roles in medical health care implementation locality for treatment and control of diseases in nearly, all cultures and civilizations specially developing countries of the world because of their effectiveness, affordability, availability, low toxicity and acceptability (Akharaiyi and Boboye 2010; Tiwari *et al.*, 2011a). Further, among all three extracts, those of DMSO

and methanol from leaves of both local fruiting plants were found to have a comparatively higher potency than water extracts but still there are variations in the antimicrobial potency (inhibitory zones) and MIC values of the plants extracts. These variations could be attributed to the varying presence pattern and combination of active phytochemicals, varying concentrations, and also depending on differences in growth rate of the tested organisms, nutritional requirements, temperature and inoculum size acceptability (Gaill and Jon 1995; Akharaiyi and Boboye 2010). Differences in polarity among the various solvents might also be responsible for the differences in solubility of plant active principles, hence the variation in degree of activity (Idris *et al.*, 2009).

Table 3. Minimal inhibition concentration of extracts from leaves of *Mangifera indica* and *Morus alba*.

Microbial pathogens	<i>Mangifera indica</i> (mg/ml)			<i>Morus alba</i> (mg/ml)		
	AE	DE	ME	AE	DE	ME
<i>Bacillus subtilis</i>	NT	65±1.5	55±0.53	80±0.35	45±0.28	44±1.36
<i>B. cereus</i>	NT	65±1.65	60±1.25	80±0.66	18±0.63	45±1.25
<i>Escherichia coli</i>	80.66±1.76	70±1.37	35±0.54	75±0.84	45±0.84	64±0.46
<i>Helicobacter pylori</i>	NT	50±1.45	55±1.85	40±1.27	34±1.33	32±1.23
<i>Klebsiella pneumoniae</i>	NT	36±0.45	30±0.78	80±0.55	60±1.5	48±0.75
<i>Pseudomonas aeruginosa</i>	70±1.45	63±0.56	30±1.35	60±1.85	40±1.42	15±1.35
<i>Salmonella typhimurium</i>	50±0.88	69±0.78	35±1.48	74±1.48	43±1.18	40±0.55
<i>Shigella flexenari</i>	NT	48±0.57	40±1.27	100±0.35	45±0.66	55±1.56
<i>Staphylococcus aureus</i>	76±0.88	55±0.27	13±1.64	67±0.56	38±1.75	46±1.64
<i>S. pyogenes</i>	77±0.57	58±0.27	30±1.44	84±0.44	40±0.67	50±0.67
<i>S. pneumoniae</i>	77±0.57	65±0.45	50±0.75	45±1.47	32±0.88	60±1.34
<i>Trichophyton longifusus</i>	85±0.35	75±0.48	70±1.76	90±1.27	50±1.24	30±0.76
<i>Candida albicans</i>	97±1.34	85±1.55	60±0.35	100±0.65	75±0.25	53±1.15
<i>Aspergillus flavus</i>	68±0.33	23±1.25	47±1.46	64±1.33	69±0.45	48±0.26
<i>Microsporum canis</i>	NT	70±1.45	55±0.56	NT	NT	48±0.76
<i>Fusarium solani</i>	95±0.54	45±1.75	25±1.35	NT	33±1.55	50±1.26
<i>Candida glabberata</i>	115±0.55	75±1.23	60±0.56	105±1.26	65±0.55	83±1.22

NT: not tested

### Antioxidant activities

The decrease in absorbance produced by reduced DPPH was used to evaluate the ability of test extracts to act as free radical scavengers. The DPPH results showed that all extracts from leaves of both fruiting plants had DPPH free radical scavenging activity ranging from 13.68±0.74 to 93.70±0.76 % by AE from *M. alba* and ME from *M. indica* respectively. Among all tested extracts, ME from *MI*, and DE and ME from *M. alba* were found to be significantly active against DPPH free radical with scavenging activity higher than that of standard antioxidant propyl gallate.

The scavenging effects of extracts on DPPH radical have demonstrated the hydrogen donating ability on reaction with DPPH radical. Earlier reports on similar studies also showed that the reduction mechanism of DPPH correlated with the presence of hydroxyl groups on the antioxidant molecules (Aliyu *et al.*, 2009). All extracts from leaves of both fruiting plants were also found to be active against superoxide anion radical with activity ranging from 50.23±0.43 to 95.7±0.21 % by DE from *M. alba* and AE from *M. indica* respectively. Superoxide anion RSA of AE and DE from *M. indica* and ME from *M. alba*

were not only found to be higher than that of standard superoxide anion radical scavenger propyl gallate (91.31%). Similar studies correlated the superoxide anion radical scavenging activities of plant extracts to the presence of phenols which was true for present investigation. Hence, these extracts could be used as natural sources of antioxidants against superoxide radicals to protect animals from adverse effects of O<sub>2</sub> (Al-Mamun *et al.*, 2007). From results of the metal-chelating assay, it was clear that all three test extracts from leaves of both fruiting plants inhibited the formation of ferrozine-Fe<sup>2+</sup> complex giving Fe<sup>2+</sup> chelating activity ranging from 52.76±0.23 to 96.82±0.42 % by DE from *M. indica* and ME from *M. alba* respectively. Among all extracts, ME from *M. alba* exhibited the maximum activity which surpassed the that by standard Fe<sup>2+</sup> chelating agent EDTA. Since, ferrozine can quantitatively form complexes

with Fe<sup>2+</sup>. In the presence of other chelating agents, the complex formation is disrupted with the result that the red color of the complex is decreased. Measurement of the rate of red color reduction, therefore, allows estimation of the chelating activity of the coexisting chelator radicals (Wang *et al.*, 2010). This suggested that the leave extract were electron donors and could neutralize free radicals. Considering the phytochemical screening, the DPPH and superoxide anion radical scavenging, and metal-chelating activity as indices of antioxidant activity of the extract, these findings revealed the therapeutic potential of leaves of under studied fruiting plants as a source for local natural antioxidants in controlling degenerative diseases with marked oxidative damage from reactive oxygen species or free radicals have been reported (Tiwari *et al.*, 2011b).

Table 4. Antioxidant activities of extract from leaves of *Mangifera indica* and *Morus alba*.

Sample	Extract	% Activity (mean ± SEM)		
		DPPH RSA	Superoxide RSA	Fe <sup>2+</sup> -chelation
<i>Mangifera indica</i>	AE	68.47±0.32	95.7±0.21	65.55±0.45
	DE	74.64±1.87	94.30±0.87	52.76±0.23
	ME	93.70±0.76	75.56±1.11	76.45±1.33
<i>Morus alba</i>	AE	13.68±0.74	54.65±0.89	55.40±1.23
	DE	92.24±0.85	50.23±0.43	71.12±0.70
	ME	91.55±1.06	92.12±1.86	96.82±0.42
n-propyl gallate		91.31	91.31	
EDTA				98.05

### Urease inhibition

Except AE from *M. indica* all extracts from each plant leaves inhibited the urease activity from moderate to significant in the range of 75 to 95.23 % by AE and DE from *M. alba* respectively. DE from Among potent extracts, DE from *M. alba* was found to significantly inhibited the urease activity which is comparable with that shown by standard urease inhibitor thiourea (Figure 1) Urease inhibition by these leaves extracts further explained their antibacterial activity against *H. pylori*.

These antiurease results provide a scientific basis for the traditional use of these plants crude extracts to treat diseases caused by the accumulation of urea and against gastrointestinal and urinary tract infection associated with pathogenic strains of *H. pylori*, *P. mirabilis*, *P. vulgaris*, and *U. urealyticum* (Estiu and Merz 2004; Tahseen *et al.*, 2010; Khan *et al.*, 2010). Further, these crude extracts also provide a reliable source for isolation of pure antiurease compounds at affordable prices (Tahseen *et al.*, 2010).



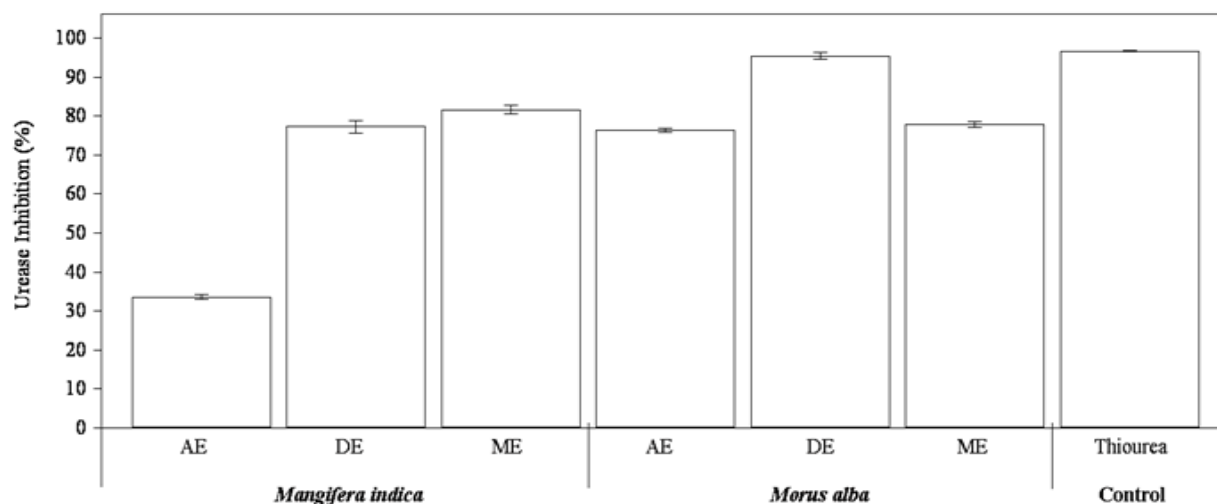


Figure. 1. Urease inhibition activity of extract from leaves of *Mangifera indica* and *Morus alba*.

### Anthelmintic activity

The assay was performed on adult earth worms (*P. pasthuma*) due to anatomical and physiological resemblance with intestinal round worms parasite in soil and as well as their easy availability. From results shown in Figure 2, it is clear that all test leaves extract from both fruiting plants caused paralysis followed by the subsequent death of the worms in period ranging from 13.5 /26.87 to 39/60.06 min for paralysis and death (P/D). The time taken by ME from *M. indica* and *M. alba* for paralysis and death (13.5/26.87 and 14.05 / 34.45 mint respectively) of parasite is least among all tested extract as well as standard drugs, albendazole (30.45/52 min) and less than piperazine citrate (14.35/36 min), thus signifying the highest efficacy (Figure 2). While the time taken by AE from both *M. indica* and *M. alba*, and DE from *M. alba* for paralysis and death is 35.8/53.04, 39/50.06 and 31.22/54.17 min respectively which are higher than that of all tested extract and standard drugs thus suggesting the least activity against helminthes parasites. On the other hands, only DE from *M. indica* took 22.2/37.76 min for paralysis and death respectively suggesting their potency higher than albendazole and less than piperazine citrate (Figure 2). Several earlier studies

revealed the *in vitro* anthelmintic activities of flavonoids, saponins, steroids, sugars, terpenoids, alkaloids, tannins, saponins and phenols extracted from plants. Such investigations are true for *in vitro* anthelmintic activities on extracts from under studied plants. Moreover, the length of time taken for paralysis and death may be dependent on concentrations and the number of potent phyto-constituents present in the plant, jointly or separately. As control anthelmintic drug piperazine citrate by increasing chloride conductance of worm muscle membrane produces hyper polarisation and reduced excitability that leads to muscle relaxation and flaccid paralysis. Similarly, among the screened phytochemicals, the possible mechanism of action of tannins and polyphenolics might interfere with energy generation by uncoupling oxidative phosphorylation by binding to glycoprotein on the cuticle of the parasite and may cause death. The main biological activity ascribed to saponins was their membrane permeabilizing and pore formation, which is similar to two conventional anthelmintic drugs such as praziquantel and toltrazuril (Kulkarni *et al.*, 2010; Tiwari *et al.*, 2011b). Alkaloids may act on central nervous system and caused paralysis of the earthworm (Roy 2010).

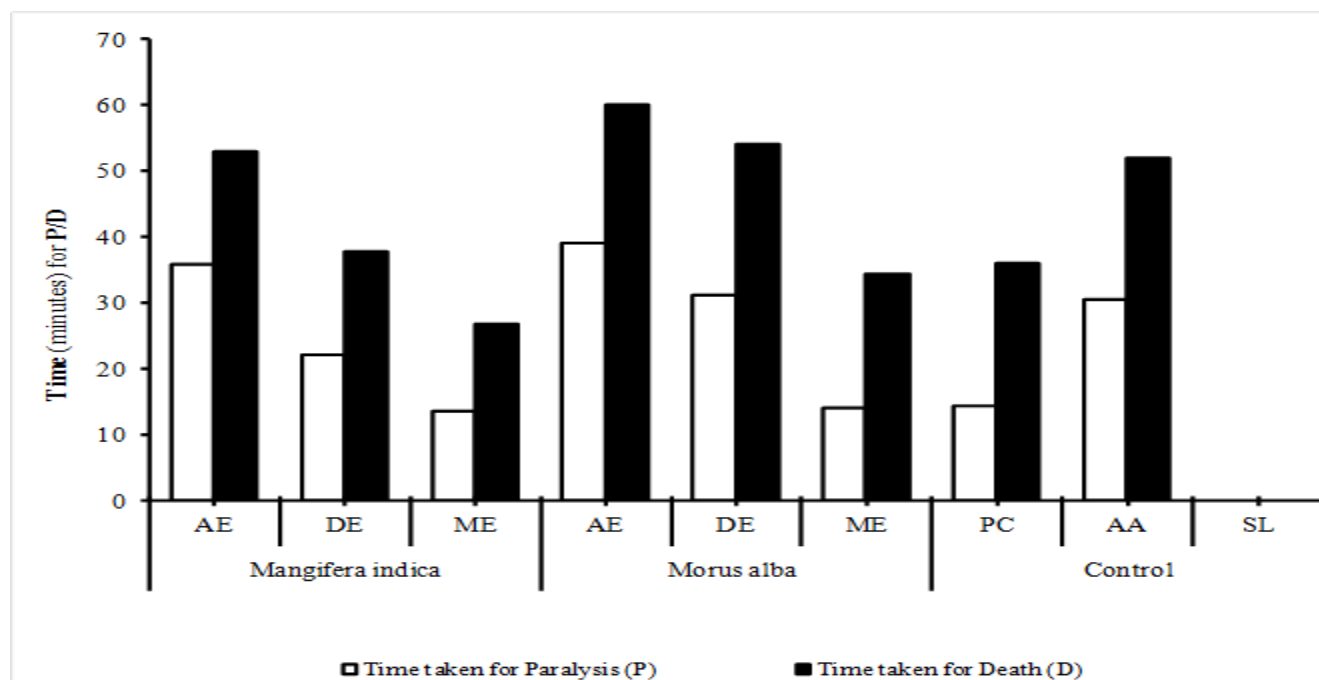


Figure. 2. Anthelmintic activity of extract from leaves of *Mangifera indica* and *Morus alba*.  
PC: Piperazine citrate; AA: Albendazole; SL: saline

## CONCLUSIONS

It can be concluded that leaves from indigenous fruiting plants *M. indica* and *M. alba* that considered as waste were found to contain a wide variety of antibacterial, antifungal, antioxidant, urease inhibitory and anthelmintic compounds. The broad spectra of antimicrobial and antioxidant, urease inhibition and anthelmintic activities displayed by these extracts explain that the purified components may have even more potency. The potent activity of extracts warrants further investigation and rapid development to add to the list of currently available pharmacological agents. Certainly, this may improve the efficacy and quality at affordable prices to decrease morbidity and mortality. The present findings provide scientific justification for the ethnomedicinal use of selected plant's leaves for treatment and controlling of infectious and degenerative diseases especially in less developing and third world countries, where infectious diseases caused by bacteria, fungi and parasites, and degenerative diseases are major health concern due to unavailability and unaffordability of modern treatments. These results also validate the traditional use of these extracts for the treatment of infectious and degenerative diseases.

## CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

## AUTHORS CONTRIBUTIONS

All the authors contributed equally to this work.

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