African Journal of Biotechnology Vol. 10(1), pp. 97-102, 3 January, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB10.852
ISSN 1684–5315 ©2011 Academic Journals

Full Length Research Paper

Phytotoxic, Antibacterial and Haemagglutination activities of the aerial parts of *Myrsine africana* L.

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Accepted 13 October, 2010

The crude methanolic extract and various fractions derived from the aerial parts of *Myrsine africana* were screened *in vitro* for possible phytotoxic, antibacterial and haemagglutination activities. Moderate phytotoxic activity (31.25 %) was observed against *Lemna minor* L at 1000 µg/ml by chloroform fraction ($CHCl_3$). The crude methanolic extract and $CHCl_3$ fraction showed good antibacterial activity against *Klebsiella pneumoniae* (MIC_{50} = 2.45 and 2.1 mg/ml respectively). The crude methanolic extract and other fractions showed moderate activity against tested bacterial strains. The $CHCl_3$ and aqueous fractions showed no activity against *Escherichia coli*. Similarly, the ethyl acetate (EtOAc) and butanol (BuOH) fractions were found to be non active against *Bacillus pumilus* and *Enterobacter aerogenes*, respectively. Moderate haemagglutination activity was observed against human red blood cells (RBCs) of blood group AB^- by crude methanolic extract and $CHCl_3$ fraction and against AB^+ by aqueous fraction, respectively. The plant specie can be a source of antibacterial agent(s) and phytolectins.

Key words: *Myrsine africana*, phytotoxicity, haemagglutination, antibacterial and MIC_{50}.

INTRODUCTION

In past decades, higher plants are well recognized for their ability to produce a wide spectrum of natural products with interesting bioactivities (Liu et al., 2007; Morita et al., 2006; Pepeljnjak et al., 2005; Tiew et al., 2003). *Myrsine africana*, locally called Babrang, belongs to the family *Myrsinaceae,* a large family of about 35 genera and nearly 1000 species, widespread mainly in the tropical and subtropical regions (Nasir and Ali, 1979), Traditionally, the plant is used as fragrance in tea, spices, carminative, appetizer and flavoring agent. Its fruits are edible and locally used as an anthelmintic (Zabta et al., 2003; Kokwaro, 1993; Beentje, 1994; Desta, 1995) for the treatment of diarrhea, rheumatism, toothache, pulmonary tuberculosis and relieving hemorrhage (Zhong, 1985). The alcoholic extract of twigs and leaves of the plants possesses significant inhibitory activities against walker intramuscular carcinosarcoma in rates (Kupchan et al., 1969). Preparations from the mixture of dried fruits and leaves of *M. africana* in water has shown 77% efficacy against *Haemonchus*, *Trichostrogylus* and *Oesophagostomum* spp. (Gathuma et al., 2004). Extracts of leaves and fruits of *M. africana* and fruits of *Rapanea melanophloeos* were not efficacious against *Haemonchus contortus* in sheep (Githiori et al., 2002). The ethanolic extract of the fruits showed lethal action against tape worms, while ethereal, alcoholic and aqueous extracts had a marked purgative activity in albino rats (Kakrani et al., 1983). Emodin and 2-hydroxychrysophanol have been isolated from ethanolic extract of the roots of the plants (Xiao-hua and Mc Laughlin, 1989). Similarly, saponin isolated from the leaves of *Maesa lanceolata* has shown virucidal, haemolytic and anthelmintic activities (Apers et al., 2001; Bagalwa and Chifundera, 2007). Embelin isolated from *R. melanophloeos* acts as an anti feedant to *Schistocerca gregaria*, and is larvicidal to

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Abbreviations: EtOAc, Ethyl acetate; **BuOH,** butanol; **DMSO,** dimethyl sulfoxide.

Aedes egyptii larvae (Midiwo et al., 1995). Anti-fertility activity of *Embelia ribes* have also been reported (Arora et al., 1971). The seed extract of the *Rapanea guinensis* Aubl gave non specific agglutinating reactions with human bloods (Schertz et al., 1960). Based on the reported literature of *M. africana* and other plants of the family, we screened *M. africana* for possible pharmacological/biological activities.

MATERIALS AND METHODS

Plant material

Aerial parts of *M. africana* (*Myrsinaceae*), were collected from Hazara division, in December, 2007 – January, 2008. The plant was identified by Professor Dr. Habib Ahmad, Plant Taxonomist, Hazara University, KPK, Pakistan.

Extraction

The extraction of crude methanolic extract from the plant material was carried out as per Ahmed et al. (2009). The shade dried plant materials were chopped into small pieces and grinded to fine powder by using electric grinder. The powdered plant material (7.6 kg) was soaked in commercial grade methanol for 15 days at room temperature and was subjected to occasional shaking. After 15 days, methanol soluble materials were filtered off. All filtrates were combined and concentrated under vacuum at 40°C using a rotary evaporator till a blackish crude extract of about 800 g was obtained.

Fractionation

The crude methanolic extract (750 g) was suspended in distilled water (400 ml) and partitioned with *n*-hexane (3 x 400 ml), $CHCl_3$ (3 x 400 ml), EtOAc (3 x 400 ml) and BuOH (3 x 400 ml) to yield *n*-hexane (50 g), $CHCl_3$ (45 g), EtOAc (255 g), BuOH(190 g) and aqueous (210 g) fractions. About 50 g of crude methanolic extract was reserved for pharmacological/biological screenings.

Phytotoxic activity

The phytotoxic activity of the test samples (methanolic crude extract and fractions) were screened against *Lemna minor* L. (McLaughlin et al., 1991), available at the department of Botany, University of Peshawar. Stock solutions of the test samples were prepared in methanol at concentration of 20 mg/ml and E-medium was prepared for the growth of *L. minor*. 10, 100 and 1000 µg/ml from the stock solution were introduced into three flasks, one for each sample and left at room temperature till the organic solvent was evaporated. 20 ml of the E-medium and sixteen healthy plants with a rosette of three fronds were added to all flasks and incubated at 28 ± 1°C for seven days. Paraquat at a concentration 0.015 µg/ml was used as standard growth inhibitor and three flasks containing E-media and *L. minor* L only. Results were noted after seven days of incubation.

Antibacterial activity

Determination of percent inhibition

The crude methanolic extract and various fractions of *M. africana* were sc*reened for possibl*e antibacterial activity against various human pathogens including *Escherichia coli*, *Pseudomonas aeruginosa, Staphylococcus aureus, Staphylococcus epidermidis, Salmonella typhi, Bacillus pumilus, Klebsiella pneumoniae, Streptococcus pneumoniae and Enterobacter aerogenes* as per our reported procedure (Ahmed et al., 2009). Test organisms were inoculated to 10 ml nutrients broth (Sigma-Aldrich, Germany) and incubated at 37°C. After 24 h, 0.6 ml from the test organism culture was added to 60 ml of molten agar at 45°C, mixed and transfered to a sterile petri dish (for the 9 cm petri dish, 0.2 ml of the culture was added to 20 ml of agar). The agar plates were allowed to harden and wells were dug in the plates at equal distance. Agar plugs were removed from the plates to open the wells. Stock solutions of the samples were prepared at concentration of 3 mg/ml in sterile dimethyl sulfoxide (DMSO < 1%); 100 µl of the test samples were loaded to their respective wells. Amoxicillin and DMSO (<1%) were used as positive and negative controls, respectively. For better diffusion of the test samples to media, the plates were left for two to three hours undisturbed inside the laminar air flow, and then incubated for 24 h at 37°C. The zones of inhibition were measured (mm).

Determination of minimum inhibitory concentration (MIC)

After recording the zone of inhibitions, experiments were performed to determine the $MIC_{50,}$ using slightly modified procedure of Banso (2009). This was performed by taking 4 ml of nutrient broth in sterile test tubes inoculated with 18 - 24 h old culture of the test organisms. 300, 500, 700 and 900 µl from the stock solution (3 mg/ml) of the samples were poured into the test tubes containing test pathogens. After 24 h of incubation at 37°C, results were recorded based on the percent clarity of the test tubes.

Haemagglutination activity

Haemagglutination activity of the crude extract and fractions was tested against human erythrocyte blood groups ABO (Naqvi et al., 1992). Stock solution of the test sample was prepared at concentration of 1 mg/ml and each solution was serially diluted. Fresh blood was collected from healthy persons, centrifuged and the erythrocytes were separated. 2% erythrocyte suspension was prepared in phosphate buffer (pH 7.4) of all blood groups. 1 ml of the test sample dilution was taken with 1 ml of 2% erythrocyte and incubated at 25°C. After incubation, the results were noted. Smooth button formation in bottom indicated negative activity, while a rough granular deposition at bottom showed positive activity. The intensity of haemagglutination was determined from the extent of deposition.

RESULTS AND DISCUSSION

In Pakistan, the rural population still mainly depends on the traditional/indigenous system of medicine for their health related matters (Khattak et al., 1985). Keeping in view the traditional/medicinal uses of the plant and previous work done, we carried out the *in vitro* biological screening of the aerial parts of M*. africana.*

Phytotoxic activity

Herbicides of the plants' origin are friendly to the environment, and this necessitates their search. The

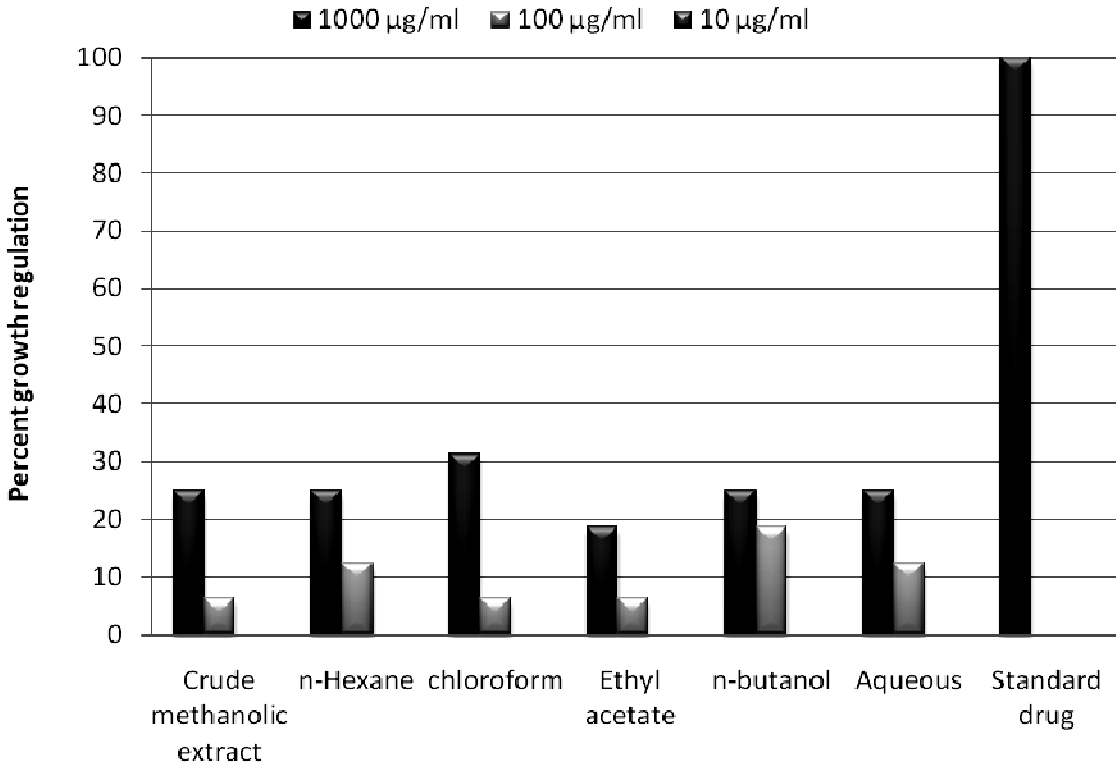

Figure 1. Phytotoxic activity of the crude methanolic extract and various fractions of *M. africana* against *L. minor*. Paraquat at a concentration 0.015 µgm/ml was used as standard drug.

phytotoxic results of the plant crude methanolic extract and various fractions against *L. minor* are summarized in Figure 1. The methanolic extract showed (25 and 6.25%), *n*-hexane (25 and 12.50%), $CHCl_3$ (31.25 and 6.25%), EtOAc (18.75 and 6.25%), BuOH (25 and 18.75%) and aqueous (25 and 12.5%) growth inhibition at concentrations of 1000 and 100 µg/m, respectively. While no phytotoxic activity was observed at concentration of 10 µg/ml.

Antibacterial activity

Crude methanolic extract and various fractions of *M. africana* were screened against the said test pathogens for possible antibacterial activity. The results are summarized in Figure 2. Order of the antibacterial activity of the crude methanolic extract and fractions against *E. coli* crude extract (60.86%) > *n*-hexane = EtOAc (52.17%) > BuOH (47.82%) > $CHCl_3$ and aqueous fractions shows no activity against *E. coli*. Against *S. epidermidis*; crude extract (39.39%) > *n*-hexane = aqueous (36.36%) > $CHCl_3$ = BuOH (33.33%) > EtOAc (30.30%). The order of activity against *S. typhi* was: crude extract (53.57%) > aqueous (52.57%) > BuOH (50.0%) > EtOAc (46.42%) > *n*-hexane (42.85%) > $CHCl_3$ (39.28%). Against *S. pneumoniae*; crude extract (57.69%) > $CHCl_3$ (53.84%) > *n*-hexane = EtOAc = BuOH = aqueous (50.00%). Against *S. aureus*; $CHCl_3$ (60.00%) > *n*-hexane (56.0 %) > EtOAc (52.0%) > crude extract = aqueous (48.0%) > BuOH (44.0 %). The activity against *P. aeruginosa* was: crude extract (60.0%) > $CHCl_3$ = BuOH (56.0 %) > *n*-hexane (52.0%) > EtOAc = aqueous (48.0%). Against *K. pneumoniae*: crude extract = $CHCl_3$ (68.42%) > *n*-hexane (63.15%) > EtOAc = aqueous (57.89%) > BuOH (47.36%). Against *B. pumilus*: crude extract (54.83%) > aqueous (51.61%) > BuOH (48.38%) > *n*-hexane = $CHCl_3$ (45.16%), while the EtOAc fraction of plant extract shows no activity. The activity of the test samples against *E. aerogenes* was in order of: crude extract (40.62%) > *n*-hexane = $CHCl_3$ (37.50 %) > EtOAc (34.37%), while BuOH and aqueous fractions were found inactive against *E. aerogenes*. From the above results, it is revealed that crude methanolic extract, $CHCl_3$ and *n*-hexane fractions showed good antibacterial activity against *K. pneumoniae*, while other fractions showed moderate to low and no activity against the tested pathogens. The lower the MIC_{50} values, the higher will be its potency and vice versa. The MIC_{50} values (mg/ml) of the crude methanolic extract and all fractions were calculated from three separate reading by using a Microsoft XL sheet, which are summarized in Figure 3. Interestingly, highest activity (100%) was displayed by the $CHCl_3$ fraction against *P. aeruginosa,* *n*-hexane fraction against *K. pneumoniae*

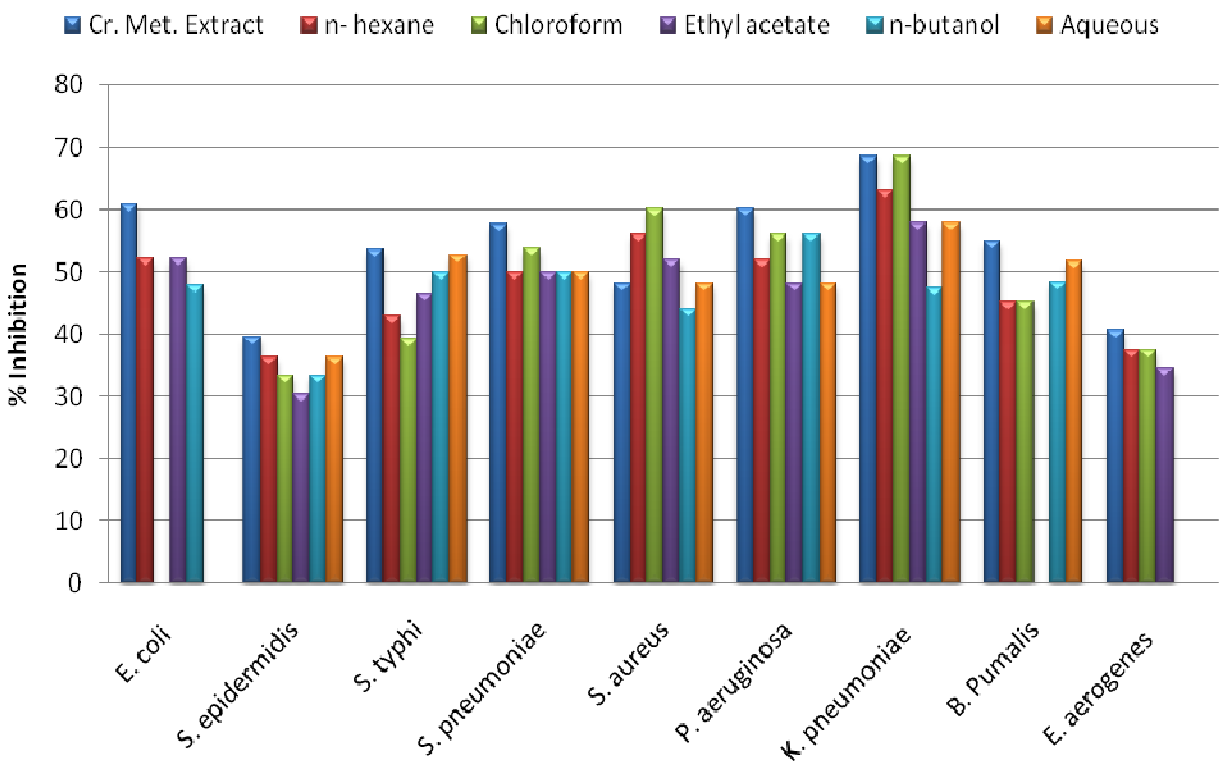

Figure 2. Antibacterial activity of the crude methanolic extract and various fractions of *M. africana.*

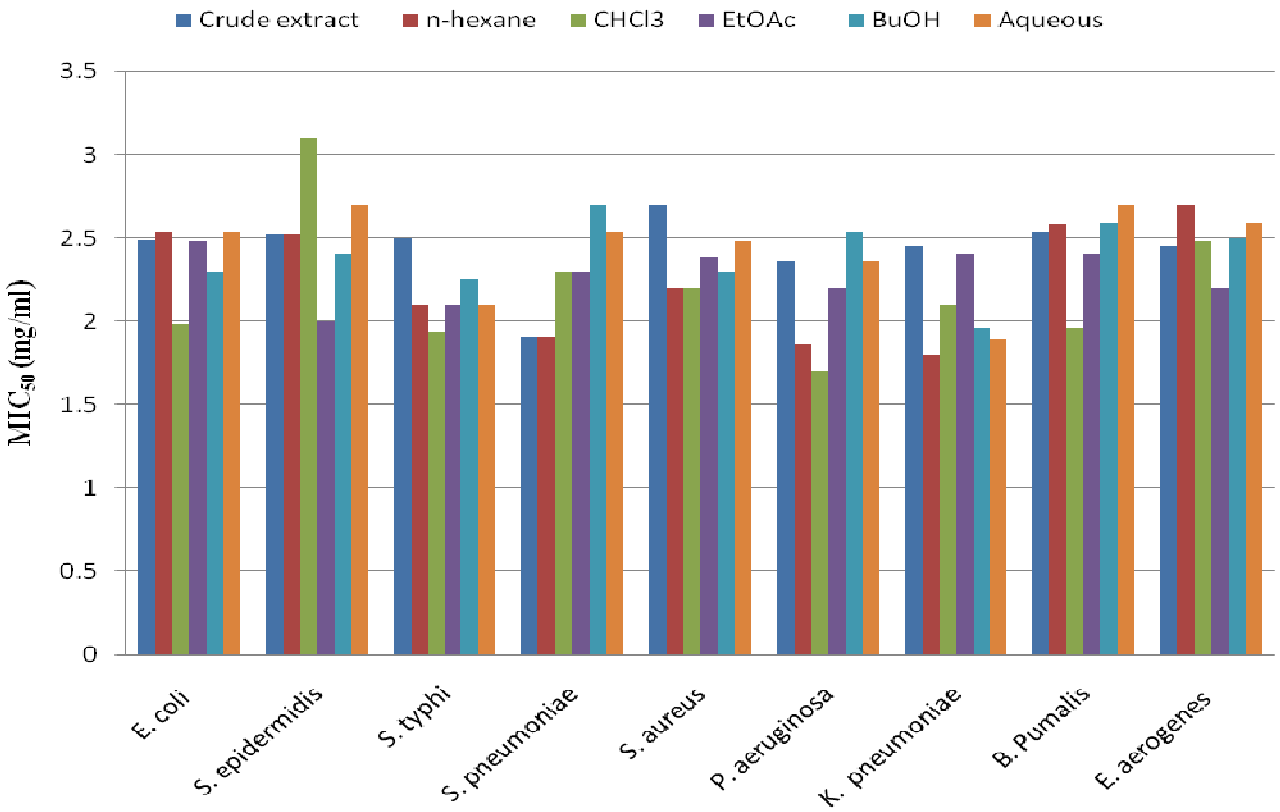

Figure 3. MIC_{50} values (mg/ml) of the crude methanolic extract and various fractions of *M. africana.*

Table 1. Haemagglutination activity of the crude methanolic extract and various fractions of *M. africana* at various dilutions.

Blood group	Crude methanolic extract				*n*-hexane				$CHCl_3$				EtOAc				BuOH				Aqueous			
	1:2	1:4	1:6	1:8	1:2	1:4	1:6	1:8	1:2	1:4	1:6	1:8	1:2	1:4	1:6	1:8	1:2	1:4	1:6	1:8	1:2	1:4	1:6	1:8
AB^-	++	+	-	-	-	-	-	-	++	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
B^-	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
A^-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
B^+	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
A^+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
AB^+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	++	+	-	-
O^+	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-
O^-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-

-, No activity; +, low activity; ++, moderate activity; +++, strong activity.

and *S.pneumoniae.* The research can further be proceeded to isolate the natural bioactive anti-bacterial compounds from the plant extracts and its fractions.

Haemagglutination activity

Certain plants have been found to contain haemagglutinins in their different parts, which can be extracted and used for the production of blood typing reagents in the same way as agglutinins from animal sources. The plant source agglutinins have advantage over the animal sources because they are economical and available in large quantities. Each test samples, crude methanolic extract and fractions of *M. africana* with different dilutions was screened for possible haemagglutination activity against human erythrocytes of all blood groups (ABO). The results showed that crude methanolic extract and $CHCl_3$ have moderate ($^{++}$) haemagglutination effect against AB^- blood group and aqueous fraction have moderate activity against O^+ blood groups at higher concentrations (1:2). Weak ($^+$) activity was also observed against the erythrocytes of other blood groups at dilution of 1:2 and 1:4. The *n*-hexane and EtOAc fractions showed no haemag-glutination activity against human red blood cells of all blood groups. Results are summarized in Table 1. The present investigation reveals that that the plant contains lectins and could be a useful source of phytolectins. It is concluded that plant species could be a potential target for activity guided isolation of antibacterial and phytotoxic agent(s). The plant can also be a source of phytolectins.

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