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EVALUATION OF ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY OF CRUDE METHANOL EXTRACT AND ITS FRACTIONS OF MUSSAENDA PHILIPPICA LEAVES

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ABSTRACT

Aim: *To evaluate the antimicrobial and antioxidant activities of crude methanol extract and its fractions of the leaves of Mussaenda Philippica.*

Methods: *MIC through disc diffusion methods and MBC are two methods to evaluate the antimicrobial activities. For studying the antioxidant activity the free radical scavenging was studied in vitro method by measuring DPPH, Hydrogen peroxide scavengering activity, Superoxide free radical (O2) and Nitric oxide (NO) free radical scavenging activity measured by considering standard antioxidant e.g. ascorbic acid.*

Results: *The test compounds like different fractions i.e. chloroform, methanol and ethyl acetate and crude methanol extract produced significant effect both in antioxidant as well as antimicrobial activities. Different microorganisms namely. E. faecalis, S. aureus, A. baumannii, E. coli, P.merabilis, P.aeruginosa are responded in higher concentration all the test compounds. Different fungal strains like C.albicans and A. niger are not inhibited by different test compounds.*

Conclusions: *The results revealed that the crude methanolic extract and different fractions like methanol and ethyl acetate are produces remarkable antimicrobial and antioxidant activities. It may assume that the activity of the test compound may be due to the active compounds such as flavonoids, terpenes, alkaloids and saponins.*

KEYWORDS: *Musa Philippica, Antimicrobial, Antioxidant, MIC*

Article History

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INTRODUCTION

At present natural substances are considered as a source of motivation for discovering a new drug. Plant derived compounds/ substances having a significant contribution towards human health and well being. [1] It is known from literatures that most of the traditional medicines obtained from natures specifically from plant materials. Plant materials are easily available in rural areas. Due to readily available of resources i.e. plants/ herbs the medicines in rural belt are cheaper than the alternative medicines like modern medicines. Medical plants and their by products (secondary metabolites) are the oldest health-care products. The importance of the herbal medicines is highly demanded in health care systems not only in developing countries but also in many developed countries [2]. The herbal medicines obtained from natural sources having no side effect along with better therapeutic effect. Herbal medicines have wide therapeutic actions because of the safety profile. From statistical report it is known that approximately 80% of the population in world rely on traditional medicine

for their primary health care and play an important role in the health care system. World Health Organization (WHO) is promoting, encouraging and facilitating the research area on herbal medicines because of the effective use of herbal medicine in developing countries for health programs [3]. Different biological activities like anti microbial, anti oxidant, sedative and anxiolytic effects of the plant extracts may be due to presence of the active compounds. Consequently, due to some other biological activities on the same time make excellent leads for new drug development [4].

Mussaenda philippica 'Aurorae' (named after Dona Aurora, wife of a former President of the Philippines). It is an ornamental plant in tropical areas. It is reported that the plant produced Hepatoprotective activity of two iridoids from. Mussaenda 'dona aurora' (sepals) has been investigated for its hepatoprotective and antioxidant activities. The highest activity was observed in the ethyl acetate fraction. [5, 6]. From different literature it was found that the methanol extract of *M. frondosa* leave produced normalizing activity against cold immobilization stress, it also reported that some biochemical transmitters also changed the transmitters are norepinephrine (NE), dopamine (DA), 5-hydroxy tryptamine (5-HT), 5-hydroxy indole acetic acid (5-HIAA), and enzyme monoamine oxidase (MAO).

The presence of bioactive compounds like iridoid glucosides, mussaenoside and shanzhiside methyl ester has been reported in different species of *Mussaenda* [7, 8,.] However, as far as the activity is concerned, there are few reports available regarding antibacterial, antifungal and antioxidant activity. Thus, the aim of the present study was to investigate the antimicrobial and antioxidant effect of crude methanol extract and different fractions like chloroform, ethyl acetate and methanol fraction of crude methanol extract of leaves of *M. Philippica*.

MATERIALS AND METHODS

Plant Material

M. Philippica leaves were collected from Puri district of Odisha, India. The leaves were authenticated by Dr. Chinmay Pradhan, Department of Botany, Utkal University, Odisha. The leaves were collected in large quantity for further processing. The raw leaves were processed step wise i.e. first the leaves are washed in running because of water may remove adhering soil and dirt particles. In second step the leaves are kept for drying i.e. shade dry. In IMT Pharmacy College, Puri, Odisha a voucher specimen was deposited for further correspondence. In third step the dried leaves were powered through mechanical grinder and stored in airtight, non-toxic polyethylene bags until used.

Preparation of Extract and Fractions

The powdered *M. Philippica* leaves of was extracted with ether in 70^0 C for 3 days for to defeating the materials after that the plant materials were macerated in different solvents based on the polarity with constant stirring. The solvent incorporating the extractives were filtered and the marc pressed to squeeze out residual extractives. This process was repeated at least thrice time for complete the extraction. For final products the extracts are evaporated generally it will be reduced to $1/8^{th}$ of its original volume by using rotary evaporator with a constant temperature i.e. 45 °C and obtained the yield. Chloroform, ethyl acetate and methanol are used as solvent for extraction. [9] Different fractions were obtained after using different solvents then the fractions are concentrated followed by dry and preserved. Chemical tests like glycosides, alkaloids, favonoids, saponins, carbohydrates, tannins, phenolic compounds, protein, and fats are performed in the compounds obtained through fraction and extract.[10] The test substances were prepared by using 10% w/v in normal saline along with 0.1% propylene glycol.

Evaluation of Antimicrobial Activity

Disc Diffusion Method

Disc diffusion test for the extracts was carried out for antimicrobial activity. The test compound for antibacterial activity was carried out by cup-plate method. Cups or discs with standard diameters are made in the nutrient agar medium. The test compounds were poured inside the discs and for result observations the diameter regarding zone of inhibition was measured. For antibacterial activity different strains like some gram positive microorganisms *S. aureus, B. subtilis* and some gram negative *E. coli, P. aeruginosa* organism are selected following agar diffusion method [11, 12]. Different test organisms were again cultured by using nutrient agar medium. 37±1^{0}C for 24 hours is the procedure for incubation then they were stored in refrigerator. A loopful stock culture is used for bacterial inoculum then transferring it to the nutrient broth (100ml) in a control flask (250ml). The flasks were incubated at 37±1^{0}C for 18 hours before starting the experimentation. Solution of the test compounds were prepared as per the guidelines i.e. dissolving 10mg of each in 10ml of dimethyl formamide (analytical grade) (1000 mg/ml conc.). A reference standard from gram positive and gram-negative bacteria was made by dissolving accurately weighed with quantity of Ampicillin (100 µg/ml) i.e. standard for Gram positive and Gram negative bacteria. Griseofulvin an antifungal agent (20 µg/ml) is used for antifungal activity.

Minimum Inhibitory Concentration (MIC) Method

MIC method is another method to evaluate the antimicrobial activity on test compounds like extracts and fractions. It proved the efficacy against different microorganisms strain. Highest plant extract dilution retains an inhibitory effect against the growth of a microorganism is known as MIC. M7-T2 publication of the National Committee for Clinical Laboratory Standards is followed for the experiment[13]. The plant extract and fractions were subjected to a serial dilution using sterile nutrient broth medium as a diluents. The two different strength of the plant extract and fraction were inoculated with 20 µl of an individual microorganism. 37°C for 24 h are inoculated for each dilution. The highest dilution of the plant extract/ fraction retained its inhibitory effect resulting in no growth (absence of turbidity) of a microorganism. At the same time a control experiment conducted in parallel to study the impact of the solvent itself (without plant components) on growth of the nine test organisms. Each solvent those are tested was diluted in a similar pattern with sterile nutrient broth.

Anti-Oxidant Activity

The antioxidant activity of the test compound those are obtained from methanol extract was determined by in vitro models. The in vitro methods include Diphenyl-picryl-hydrazyl (DPPH) radical, Superoxide free radical (O_2), Peroxide radical (H_2O_2), and Nitric oxide (NO) free radical scavenging activity with reference to standard antioxidant ascorbic acid[14].

DPPH Free-Radical Scavenging Activity

The free radical scavenging activity of the metabolite, based on the scavenging activity of the stable 1,1- diphenyl-2-picrylhydrazyl (DPPH) free radical was assayed according to the protocol [15]. Briefly, different concentrations of the extracts and ascorbic acid were prepared. Various concentration of test solution in 0.1ml was added to 0.9 ml of 0.1 mM solution of DPPH in methanol. Control was 100µl methanol and 100µl DPPH solution. After 30 minute of incubation at room temperature, the reduction in the number of free radical was measured, reading the absorbance at 517nm. Ascorbic acid was used as reference standard. The scavenging activity of the samples corresponded to the intensity of quenching DPPH. Ascorbic acid was used as reference standard. The compounds with antiradical activity changed color as yellow from the purple-blue.

Quantitative Determination of the DPPH Radical Scavenging Activity

The antioxidant activity of the M. Philippica methanolic extract and different fractions were evaluated spectrophotometrically following the DPPH method. Different concentrations of the extracts and fractions 100, 200 and 500 µg/ml) were prepared and mixed 1 ml of them with 2 ml of a freshly prepared DPPH solution (0.01mM); then, each particular sample was mixed thoroughly and kept in the dark for 30 minutes, at room temperature. After that, each mixture was tested for the DPPH radical scavenging activity by reading the absorbance at 517 nm on a UV-VIS spectrophotometer. As blank was used a solution prepared by mixing 1 ml of methanol with 2 ml of the DPPH solution (0.01mM) and reading at the same wavelength. In addition, to eliminate the absorbance of the crude extracts at this wavelength, blank samples were prepared with 1 ml of each extract and 2 ml of methanol. The antioxidant activity percentage was calculated following the formula:

$$\text{Antioxidant activity } (\%) = [(AC - AE) / AC] \times 100$$

Where AC is the absorbance of a DPPH solution without extract, AE is the absorbance of the tested extract and fractions, which is equal to the absorbance of the plant extract plus the DPPH (0.01mM) minus the blank extract absorbance. As standard ascorbic acid at different concentration 5-25 µg.ml-1 was used. The EC50 value, defined as the concentration of the sample leading to 50% reduction of the initial DPPH concentration, was calculated from the linear regression of plots of concentration of the test extracts against the mean percentage of the antioxidant activity[16].

Superoxide (O_2) Free-Radical Scavenging Activity

Measurement of superoxide anion (O_2) scavenging activity of extracts and fractions was based on the method with slight modification 24. O2- radicals are generated non-enzymatically in Phenazine methosulphate–Nicotinamide adenine dinucleotide phosphate (PMS–NADH) systems by the oxidation of NADH and assayed by the reduction of NBT. In this experiment, the superoxide radicals were generated in 1 mL of Tris- HCl buffer (16 mM, pH 8.0) containing nitro blue tetrazolium (NBT) (50 µM) solution and NADH (78 µM) solution. The reaction was started by adding PMS solution (10 µM) to the mixture. The reaction mixture was incubated at 25°C for 5 min, and the absorbance at 560 nm in a spectrophotometer was measured against blank samples. Decreased absorbance of the reaction mixture indicated increased superoxide anion scavenging activity. The percentage inhibition of superoxide anion generation was calculated using the following formula:

$$(\%)\ I = (A0 - A1) / (A0) \times 100$$

Where A0 was the absorbance of the control and A1 was the absorbance of extract and the standard compound.

Peroxide Free Radical (H_2O_2) Scavenging Activity

Scavenging of H_2O_2 by the extract and fractions was determined. One millilitre of C. siamea flower extract solution [prepared in phosphate buffered saline (PBS)] was incubated with 0.6 ml of 4mM H_2O_2 solution (prepared in PBS) for 10 min. The absorbance of the solution was measured at 230 nm against a blank solution containing the extract without H_2O_2[17]. The concentration of H_2O_2 was spectrophotometrically determined from absorption at 230 nm using the molar absorptivity.

Nitric Oxide Free Radical (No) Scavenging Activity

Nitric oxide (NO) was generated from sodium nitropruside (SNP) and measured by Greiss reaction. SNP in aqueous solution at physiological pH instinctively generates NO, which intermingles with oxygen to generate nitrite ions that can be anticipated by Greiss reagent (1% sulphanilamide, 2% phosphoric acid and 0.1% naphthyl ethylene diamine dihydrochloride). Scavengers of NO with oxygen leading to reduced production of NO. SNP (5 mM) in phosphate buffer saline (PBS) was mixed with different concentration of (100-500 µg/mL) drug dissolved in suitable solvent and incubated at 25oC for 150 min. The above samples were reacted with Greiss reagent. The absorbance of chromophore created during diazotization of nitrite with sulphanilamide and following coupling with naphthyl ethylene diamine was read at 546 nm and referred to the absorbance of standard solution of potassium nitrite treated in the same way with Greiss reagent[18].

Statistical Data Analysis

Results were expressed as mean ± SEM. All the results were analyzed by One-way Analysis of Variance (ANOVA) followed by Dunnett's test. The level of significance was set at $P<0.05$.

RESULTS

The phytochemical screening (Table. 1) of the different fractions and crude methanolic extact of *M. Philippica* revealed the presence of glycosides, steroids, flavonoids, terenoids, saponins and reducing sugar. The crude methanolic extracts and fractions of the plant was studied against both gram-negative, grampositive bacteria and fungus related to their zone of inhibition, MIC and MBC. The results of the antimicrobial screening of the methanol extracts and different fractions of *M. Philippica* was shown in (Tables 2 and 3). The results were recorded as presence or absence of zones of inhibition around the well as well as MIC and MBC. The inhibitory zone around the well indicated the absence of bacterial growth and it was reported as positive and absence of zone as negative. The crude methanolic extracts of leaves of *M. Philippica* Showed moderate to high antimicrobial activity against all the tested microorganisms except *A.niger* and *C.Albicans*. Crude methanolic extract and different fractions were used to assess the in-vitro antioxidant activity. The antioxidant activity of chloroform fraction was determined by different in vitro methods such as, the DPPH free radical scavenging assay and reducing power methods in (Tables 4). All the assays were carried out in triplicate and average values were considered. Antioxidant scavengering activity was studied using 1, 1—diphenyl, 2-picrylhydrazyl free radical. Various concentration of test solution in 0.1ml was added to 0.9 ml of 0.1 mM solution of DPPH in methanol. Methanol only (0.1ml) was used as experimental control. After 30 minutes of incubation at room temperature, the reduction in the number of free radical was measured, Ascorbic acid was used as reference standard. The crude methanolic extract along with chloroform, ethayl acetate methanolic, and fractions showed a concentration dependent antiradical activity by scavengering DPPH radical. Crude methanolic extract was found to be more potent compared to other fractions. The observations made in the present study showed that the extract of *M. Philippica* leaves exhibited good scavengering of H2O2 in the biological system, thus preventing the stress induced by progressive increase in malondialdehyde and other free radicals which cause oxidative damages (Tables 5). All various fractions and crude methanolic extract of these plants were capable of reducing DNA damage comparing to control. The percentage inhibition of nitric oxide generation by different fractions and extracts of *M. Philippica* leaves at different concentration were compared with standard (Tables 6). All test compounds exhibited potential inhibiting activity against NO generation. Nitric oxide is a potent pleiotropic mediator of physiological processes. The antioxidant activity of crude methanolic extract of *M. Philippica* showed highest inhibition of nitric oxide generation which is compared with standard ascorbic acid. All the data along with test standard and control compounds are present in the table i.e. ((Tables 7)

DISCUSSIONS

The different parts of the plant such as root, bark and leaves of *M. Philippica* has been used for thousands of years for its medicinal properties. It is rich in a wide variety of secondary metabolites such as glycosides, phytosterols, proteins, saponins and phytosterols. In this connection the present study on the methanolic extract and the different fractions of crude methanolic extract was conducted to evaluate the antimicrobial activity of leaves. Phytomedicines can be used for the treatment of diseases as is done in case of Unani and ayurvedic system of medicines, a natural blue print for the development of new drugs. The MIC values of those extract and fractions which gave positive results during prelim [19]. Screenings were determined by Tube Dilution Method. The antimicrobial study was conducted using different micro organisms such as *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *A. Baumannii*, *Candida albicans*, *Aspergilus Niger*. The extract and fractions of *M. Philippica* showed the higher zone of inhibition. Though the growth of *Staphylococccus aureus*, *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, Pseudomonas aeruginosa are controlled by *M. Philippica*, it indicates that they could inhibit the activity of bacteria which causes diarrhoea, polymixin and typhoid respectively. Polyphenols, including flavonoids, forms a large group of naturally occurring components of the plant kingdom and are present in every part of the plants. These compounds are of considerable interest in various fields such as food, pharmacy and medicine because of wide range of biological activities including antioxidant activity[20]. The antioxidant efficacy of phenolic compounds is chiefly due to their redox potential. These compounds are known to act as reducing agents (free radical terminators), hydrogen donors, metal chelators and singlet oxygen quenchers. Since it has been shown that phenolic compounds of plant kingdom are one of the most effective antioxidative constituents. Flavonoids are polyphenolic compounds and consist of flavones, flavonols, flavanols, flavanone and flavanonols. These compounds represent the majority of plant secondary metabolites and have shown to possess remarkable health promontory effects such as anti-inflammatory, antioxidant, antimicrobial, anticancer and others. Interception of free radicals or other reactive species is mainly by radical scavenging and is caused by various antioxidants like vitamins C and E, glutathione, other thiol compounds, carotenoids, flavonoids, etc. While at the repair and reconstitution level, mainly repair enzymes are involved. In general, peroxyl radicals cause chain reactions in lipids, proteins and DNA [22]. The high reactivity of the representative peroxyl radical shows that the possible mechanism behind the observed protection of these biomolecules by DA may be through scavenging of secondary radicals. The soluble free radical DPPH is well known as a good hydrogen abstractor yielding DPPH-H as by product. Thus, the scavenging of DPPH radicals by phenols are most of the time very effective. All the plant extracts used in this study were primarily screened against the test microorganisms by the Different Methods like Disc diffusion and MIC methods. The relative efficacy of some commonly used antibiotics were compared with plant extract discs [23]. On the basis of the results finding in the present investigation, it is concluded that the crude methanolic extract of *M. Philippica* produces highest wound healing activity plant extract and Fractions. The present study suggests that the antimicrobial and antioxidant activity can be enhanced by the use of crude methanolic extracts of *M. Philippica*.

CONCLUSIONS

Drugs from plants have a long history in both traditional and modern societies as herbal remedies or crude drugs and as purified compounds. The present study revealed that the selected Plant extract and some fractions of the crude extract produced antimicrobial and antioxidant activity with dose dependent manner. The observed activities of leave extract might be attributed to the presence of secondary metabolites such as flavonoids and phenolic compounds. The leaves can

be used to prevent oxidative damage caused by free radicals and to treat infections caused by pathogenic bacteria not to fungus. Further studies with purified constituents are needed to understand the complete mechanism of wound healing activity of the test plant. However, it needs further evaluation in clinical settings before consideration for the treatment of different disorders.

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Table 1: Preliminary Phytochemical Screening of Fractions and Extract of *M. philippica*

Constituent	CF	EAF	MF	AQF	MEMP
Alkaloids	-	-	+	+	+
Tannins	+	+	+	+	+
Triterpenoids	+	+	+	+	+
Saponins	+	+	+	+	+
Flavonoids	-	+	+	+	+
Phenols	+	+	+	+	+
Glycosides	+	+	+	+	+
Steroids	-	-	-	-	-
Carbohydrates	-	-	-	-	-

(-) Absent, (+) Present

The results of the antimicrobial screening of the methanol extracts and different fractions of *M. philippica* were shown in (Table 3 and 4). The results were recorded as presence or absence of zones of inhibition around the well as well as MIC and MBC. The inhibitory zone around the well indicated the absence of bacterial growth and it was reported as positive and absence of zone as negative. The crude methanolic extracts of leaves of *M. philippica* showed moderate to high antimicrobial activity against all the tested microorganisms except A. niger and C. albicans.

Table 2: Antimicrobial Activity of *M. Philippica* by Agar Well Diffusion Method

Sample	Conc^{n}	Zone of inhibition in(mm)									
		S.aureus	S.Epidermidis	E. Coli	P. Aeruginosa	*P. Merabilis*	*A. Baumannii*	B. Subtilis	*E. Faecalis*	C. Albicans	A. Niger
DMSO	100(µg/ml)	4.1 ± 0.72	4.3± 0.56	3.9 ± 0.5	4.1 ± 0.6	3.2 ± 0.4	4.2 ± 0.7	5.1 ± 0.7	4.2 ± 0.5	3.8 ± 0.7	3.4 ± 0.5
Griseofulvin	20(µg/ml)	NA	NA	NA	NA	NA	NA	NA	NA	31.4±2.3^{c}	30.2±2.1^{c}
Ampicillin	100 (µg/ml)	26.42±0.25^{c}	28.6±0.22^{c}	23.5 ± 0.7^{c}	29.12±0.7^{c}	30.4 ± 0.6^{c}	24.8±0.9^{c}	31.4±0.7^{c}	30.2±0.6^{c}	NA	NA
Chloroform Fraction	100 (mg/ml)	6.3±0.67	8.2±1.15	10.4 ±0.5^{b}	6.42±0.64^{a}	13.2±0.8^{c}	12.2±0.8^{c}	8.6 ± 0.6	4.6 ± 0.4	7.4 ± 0.6^{a}	4.8 ± 0.5
	200 (mg/ml)	10.2±0.46^{c}	11.42±0.34^{c}	14.2 ±0.8^{c}	16.24±0.67^{c}	18.12±0.44^{c}	15.6±0.6^{c}	14.24±0.68^{c}	14.8±0.58^{c}	7.6 ±0.4^{b}	5.1 ± 0.4
Ethayl Acetate Fraction	100 (mg/ml)	10.4±0.64^{c}	12.23±1.44^{c}	10.2 ± 0.6^{c}	14.54±0.46^{c}	12.4 ± 0.8^{c}	12.4± 0.8^{c}	8.2 ± 0.5	13.4 ± 0.8^{c}	6.8 ±0.6	4.2 ± 0.8
	200 (mg/ml)	13.8±0.66^{c}	14.24±0.48^{c}	14.24±0.66^{c}	16.26±0.62^{c}	14.24±0.68^{c}	14.4±0.9	11.89±0.48^{c}	15.2±0.68^{c}	7.2 ± 0.6^{a}	5.2 ± 0.4
Aqueous Fraction	100 (mg/ml)	14.22±0.68^{c}	16.24±0.67^{c}	16.24±2.2^{c}	16.8±0.9^{c}	14.8±0.6^{c}	18.26±0.7^{c}	12.2 ±0.5^{c}	14.2±0.7^{c}	6.8 ± 0.9^{a}	4.8 ± 0.4^{a}
	200 (mg/ml)	16.4±0.6^{c}	18.8±0.6^{c}	19.24±0.8^{c}	18.9±0.6^{c}	17.4±0.9^{c}	20.4±0.6^{c}	14.6 ±0.6^{c}	17.6±0.6^{c}	6.3 ±0.5^{b}	6.2 ± 0.4^{b}
Crude methanolic extract	100 (mg/ml)	16.8±0.6^{c}	17.6±0.9^{c}	17.8 ±0.8^{c}	18.24±0.66^{c}	17.8±0.46^{c}	18.24±0.5^{c}	12.4 ±0.7^{c}	17.4±0.6^{c}	6.6 ± 0.6^{a}	4.7 ± 0.8^{a}
	200 (mg/ml)	20.4±0.8^{c}	22.4±0.64^{c}	19.5 ±0.6^{c}	20.4±0.54^{c}	20.4±0.62^{c}	21.2±0.6^{c}	16.2 ±0.6^{c}	19.6±0.6^{c}	7.5 ±0.2^{b}	7.8 ± 0.6^{b}

Values are mean ± S.E.M. of 3 replications. S.aureus:- Staphylococcus aureus, S.epidermidis-Staphylococcus epidermidis, E.coli- Escherichia coli, P.aeruginosa- Pseudomonas aeruginosa, P. mirabilis:- Pseudomonas mirabilis, A. baumannii:- C.albicans:-Candida albicans. A. Niger:- aspergilus Niger (NA)- no measurable zone of inhibition. Aqueous

extract,methanol extract and Chloroform extracts respectively each having cocentration 300 µg per disc. significance at *p<0.05, **p<0.01. t-value denotes significance at $^{a}p<0.05$, $^{b}p<0.01$, $^{c}p<0.001$

Table 3: The MBC Regimes of the Extracts and Fractions of the Leave of *M. Philippica*

Strain	*M. Philippica* Extract and Fractions							
	CF		EAF		AF		MEMP	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
E. faecalis	4.1	4.1	3.8	10.2	4.1	22.4	4.1	10.2
S. aureus	4.2	8.8	4.1	5.1	10.2	20.5	10.2	22.9
A. baumannii	7.1	16.2	10.2	22.4	10.4	22.8	4.2	20.8
E. coli	4.1	13.5	10.4	20.5	4.1	16.2	8.8	22.2
P. merabilis	3.4	14.2	5.1	10.2	10.4	22.5	4.4	23.8
P.aeruginosa	3.1	10.2	10.2	22.4	4.2	5.1	4.2	10.4

Table 4: Determination of 1, 1-Diphenyl-2-Picrylhydrazyl (DPPH), Superoxide (O_2), Hydrogen Peroxide (H_2O_2) and Nitric Oxide (NO) Scavenging Activity of Chloroform Fraction of *M. Philippica*

Groups & Treatments	DPPH		O_2		H_2O_2		NO	
	% of Control	% Scavenging Activity	% of Control	% Scavenging Activity	% of Control	% Scavenging Activity	% of Control	% Scavenging Activity
Control	100.0 ± 4.1	00	100.0 ± 3.8	00	100.0 ± 3.9	00	100.0 ±4.2	00
Ascorbic acid								
100 µg/ml	30.2 ± 3.8c	69.8	40.2 ± 4.1b	59.8	58.6 ± 4.6	41.4	68.2 ± 3.4	31.8
200 µg/ml	16.8 ± 2.2c	83.2	28.6 ± 3.6b	71.4	48.6 ± 4.1	51.4	60.2 ± 4a	39.8
400 µg/ml	4.2 ± 2.2c	95.8	3.4 ± 2.8c	96.6	26.4 ± 1.6c	73.6	46.4 ± 3.2b	53.6
500 µg/ml	0.00	100	1.2 ± 2.5c	98.8	16.2 ± 1.2c	83.8	28.4 ± 2.5c	71.6
CF								
100 µg/ml	67.7 ± 2.6	32.3	66.4 ± 2.8	33.6	70.4 ± 3.6	29.6	74.3 ± 3.1	25.7
200 µg/ml	58.4 ± 2.2	41.6	58.6 ± 3.2	41.4	66.2 ± 3.2	33.8	64.2 ± 2.8	35.8
400 µg/ml	56.8 ± 1.8a	43.2	53.5 ± 2.4a	46.5	56.4 ± 2.9a	43.6	60.4 ± 3.1a	39.6
500 µg/ml	49.5 ± 2.8a	50.5	47.6 ± 2.8a	52.4	47.8 ± 2.2b	52.2	54.6 ± 3.3a	45.4

Values are expressed in MEAN ± S.E.M (n =3). (t-value denotes statistical significance at $^{a}p<0.05$, $^{b}p<0.01$ and $^{c}p<0.001$ respectively, in comparison to control group)

Table 5: Determination of 1, 1-Diphenyl-2-Picrylhydrazyl (DPPH), Superoxide (O_2), Hydrogen Peroxide (H_2O_2) and Nitric Oxide (NO) Scavenging Activity of Ethyl Acetate Fraction of *M. Philippica*

Groups & Treatments	DPPH		O_2		H_2O_2		NO	
	% of Control	% Scavenging Activity	% of Control	% Scavenging Activity	% of Control	% Scavenging Activity	% of Control	% Scavenging Activity
Control	100.0 ± 4.1	00	100.0 ± 3.8	00	100.0 ± 3.9	00	100.0 ±4.2	00
Ascorbic acid								

100 µg/ml	30.2 ± 3.8c	69.8	40.2 ± 4.1b	59.8	58.6 ± 4.6	41.4	68.2 ± 3.4	31.8
200 µg/ml	16.8 ± 2.2c	83.2	28.6 ± 3.6b	71.4	48.6 ± 4.1	51.4	60.2 ± 4a	39.8
400 µg/ml	4.2 ± 2.2c	95.8	3.4 ± 2.8c	96.6	26.4 ± 1.6c	73.6	46.4 ± 3.2b	53.6
500 µg/ml	0.00	100	1.2 ± 2.5c	98.8	16.2 ± 1.2c	83.8	28.4 ± 2.5c	71.6
EAF								
100 µg/ml	66.8 ± 3.4a	33.2	76.7 ± 5.4	23.3	76.2 ± 4.1	23.8	78.4 ± 6.7	21.6
200 µg/ml	56.4 ± 3.8a	43.6	62.6 ± 6.4	37.4	67.5 ± 3.8	32.5	73.4 ± 5.2	26.6
400 µg/ml	52.4 ± 3.8a	47.6	58.4 ± 4.6a	41.6	60.6 ± 4.1a	39.4	68.4 ± 3.8a	31.6
500 µg/ml	50.2 ± 2.4b	49.8	52.8 ± 3.8b	47.2	57.6 ± 3.8b	42.4	64.5 ± 4.4b	35.5

Values are expressed in MEAN ± S.E.M (n =3). (t-value denotes statistical significance at [a]$p<0.05$, [b]$p<0.01$ and [c]$p<0.001$ respectively, in comparison to control group)

Table 6: Determination of 1, 1-Diphenyl-2-Picrylhydrazyl (DPPH), Superoxide (O_2), Hydrogen Peroxide (H_2O_2) and Nitric Oxide (NO) Scavenging Activity of *M. Philippica*

Groups & Treatments	DPPH		O_2		H_2O_2		NO	
	% of Control	% Scavenging Activity	% of Control	% Scavenging Activity	% of Control	% Scavenging Activity	% of Control	% Scavenging Activity
Control	100.0 ± 4.1	00	100.0 ± 3.8	00	100.0 ± 3.9	00	100.0 ±4.2	00
Ascorbic acid								
100 µg/ml	30.2 ± 3.8c	69.8	40.2 ± 4.1b	59.8	58.6 ± 4.6	41.4	68.2 ± 3.4	31.8
200 µg/ml	16.8 ± 2.2c	83.2	28.6 ± 3.6b	71.4	48.6 ± 4.1	51.4	60.2 ± 4a	39.8
400 µg/ml	4.2 ± 2.2c	95.8	3.4 ± 2.8c	96.6	26.4 ± 1.6c	73.6	46.4 ± 3.2b	53.6
500 µg/ml	0.00	100	1.2 ± 2.5c	98.8	16.2 ± 1.2c	83.8	28.4 ± 2.5c	71.6
AF								
100 µg/ml	78.4 ± 4.6a	21.6	82.6 ± 5.4	17.4	77.4 ± 4.8	22.6	77.4 ± 5.4a	22.6
200 µg/ml	69.6 ± 5.1a	30.4	74.6 ± 4.9	25.4	68.2 ± 4.1	31.8	68.2 ± 4.8a	31.8
400 µg/ml	55.6 ±4.2b	44.4	66.4 ± 4.2b	33.6	56.6 ± 4.2	43.4	58.4 ± 4.2c	41.6
500 µg/ml	44.2 ± 4.3c	55.8	44.2 ± 4.8b	55.8	48.4 ± 3.7b	51.6	46.5 ± 3.3c	53.5

Values are expressed in MEAN ± S.E.M (n =3). (t-value denotes statistical significance at [a]$p<0.05$, [b]$p<0.01$ and [c]$p<0.001$ respectively, in comparison to control group)

Table 7: Determination of 1, 1-Diphenyl-2-Picrylhydrazyl (DPPH), Superoxide (O_2), Hydrogen Peroxide (H_2O_2) and Nitric Oxide (NO) Scavenging Activity of Aqueous Fraction of *M. Philippica*

Groups & Treatments	DPPH		O_2		H_2O_2		NO	
	% of Control	% Scavenging Activity	% of Control	% Scavenging Activity	% of Control	% Scavenging Activity	% of Control	% Scavenging Activity
Control	100.0 ± 4.1	00	100.0 ± 3.8	00	100.0 ± 3.9	00	100.0 ±4.2	00
Ascorbic acid								
100 µg/ml	30.2 ± 3.8c	69.8	40.2 ± 4.1b	59.8	58.6 ± 4.6	41.4	68.2 ± 3.4	31.8
200 µg/ml	16.8 ± 2.2c	83.2	28.6 ± 3.6b	71.4	48.6 ± 4.1	51.4	60.2 ± 4a	39.8
400 µg/ml	4.2 ± 2.2c	95.8	3.4 ± 2.8c	96.6	26.4 ± 1.6c	73.6	46.4 ± 3.2b	53.6
500 µg/ml	0.00	100	1.2 ± 2.5c	98.8	16.2 ± 1.2c	83.8	28.4 ± 2.5c	71.6
MEMP								
100 µg/ml	64.6 ± 5.8[a]	31.9	72.2 ± 4.8	10.8	84.2 ± 4.6	8.6	68.6 ± 3.4[a]	21.5
200 µg/ml	54.5 ± 4.7[a]	46.5	67.4 ± 3.9	28.7	79.6 ± 3.9	12.8	56.4 ± 3.6[a]	28.2
400 µg/ml	34.1 ±3.2[b]	64.2	58.8 ± 3.2[b]	46.4	45.7 ± 3.5	53.8	45.8 ± 4.6[c]	42.8
500 µg/ml	12.5 ± 2.3[c]	79.6	43.8 ± 2.8[b]	56.2	34.6 ± 2.6[b]	65.4	38.8 ± 2.4[c]	56.8

Values are expressed in MEAN ± S.E.M (n =3). (t-value denotes statistical significance at $^{a}p<0.05$, $^{b}p<0.01$ and $^{c}p<0.001$ respectively, in comparison to control group)

Table 8: Determination of 1, 1-Diphenyl-2-Picrylhydrazyl (DPPH), Superoxide (O_2), Hydrogen Peroxide (H_2O_2) and Nitric Oxide (NO) Scavenging Activity of Different Efractions and Crude Extract of *M. Philippica*

Groups & Treatments	DPPH		O_2		H_2O_2		NO	
	% of Control	% Scavenging Activity	% of Control	% Scavenging Activity	% of Control	% Scavenging Activity	% of Control	% Scavenging Activity
Control	100.0 ± 4.1	00	100.0 ± 3.8	00	100.0 ± 3.9	00	100.0 ±4.2	00
Ascorbic acid								
100 µg/ml	30.2 ± 3.8c	69.8	40.2 ± 4.1b	59.8	58.6 ± 4.6	41.4	68.2 ± 3.4	31.8
200 µg/ml	16.8 ± 2.2c	83.2	28.6 ± 3.6b	71.4	48.6 ± 4.1	51.4	60.2 ± 4a	39.8
400 µg/ml	4.2 ± 2.2c	95.8	3.4 ± 2.8c	96.6	26.4 ± 1.6c	73.6	46.4 ± 3.2b	53.6
500 µg/ml	0.00	100	1.2 ± 2.5c	98.8	16.2 ± 1.2c	83.8	28.4 ± 2.5c	71.6
CF								
100 µg/ml	67.7 ± 2.6	32.3	66.4 ± 2.8	33.6	70.4 ± 3.6	29.6	74.3 ± 3.1	25.7
200 µg/ml	58.4 ± 2.2	41.6	58.6 ± 3.2	41.4	66.2 ± 3.2	33.8	64.2 ± 2.8	35.8
400 µg/ml	56.8 ± 1.8a	43.2	53.5 ± 2.4a	46.5	56.4 ± 2.9a	43.6	60.4 ± 3.1a	39.6

500 µg/ml	49.5 ± 2.8a	50.5	47.6 ± 2.8a	52.4	47.8 ± 2.2b	52.2	54.6 ± 3.3a	45.4
EAF								
100 µg/ml	66.8 ± 3.4a	33.2	76.7 ± 5.4	23.3	76.2 ± 4.1	23.8	78.4 ± 6.7	21.6
200 µg/ml	56.4 ± 3.8a	43.6	62.6 ± 6.4	37.4	67.5 ± 3.8	32.5	73.4 ± 5.2	26.6
400 µg/ml	52.4 ± 3.8a	47.6	58.4 ± 4.6a	41.6	60.6 ± 4.1a	39.4	68.4 ± 3.8a	31.6
500 µg/ml	50.2 ± 2.4b	49.8	52.8 ± 3.8b	47.2	57.6 ± 3.8b	42.4	64.5 ± 4.4b	35.5
AF								
100 µg/ml	78.4 ± 4.6a	21.6	82.6 ± 5.4	17.4	77.4 ± 4.8	22.6	77.4 ± 5.4a	22.6
200 µg/ml	69.6 ± 5.1a	30.4	74.6 ± 4.9	25.4	68.2 ± 4.1	31.8	68.2 ± 4.8a	31.8
400 µg/ml	55.6 ±4.2b	44.4	66.4 ± 4.2b	33.6	56.6 ± 4.2	43.4	58.4 ± 4.2c	41.6
500 µg/ml	44.2 ± 4.3c	55.8	44.2 ± 4.8b	55.8	48.4 ± 3.7b	51.6	46.5 ± 3.3c	53.5
MEMP								
100 µg/ml	44.2 ± 3.8a	55.8	66.8 ± 4.8	33.2	56.4 ± 3.9	43.6	65.2 ± 4.8a	34.8
200 µg/ml	39.4 ± 4.2a	60.6	55.6 ± 3.8	44.4	48.4 ± 5.1	51.6	57.4 ± 4.2a	42.6
400 µg/ml	35.4 ±3.8b	64.6	47.2 ± 4.6b	52.8	38.6 ± 4.8	61.4	47.6 ± 5.2c	52.4
500 µg/ml	26.8 ± 4.4c	73.2	38.4 ± 4.2b	61.6	32.9 ± 4.6b	67.1	38.4 ± 4.4c	61.6

Values are expressed in MEAN ± S.E.M (n =3). (t-value denotes statistical significance at $^{a}p<0.05$, $^{b}p<0.01$ and $^{c}p<0.001$ respectively, in comparison to control group)