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Evaluation of antimicrobial and antioxidant properties of *Momordica dioica Roxb. (Ex Willd)*

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ABSTRACT

Momordica dioica Roxb is a perennial climbing creeper belonging to the family Curcurbitaceae and generally found in the forests of Southern India, Bengal, Maharashtra and Madhya Pradesh. In the present study the methanolic extract (MEFMD) and aqueous extract (AEFMD) of fruits of *Momordica dioica Roxb* were analyzed for the presence of different phytochemical constituents and total phenolic content. MEFMD after drying on rotary evaporator was used for screening of antimicrobial and antioxidant properties. The screening of antimicrobial activity was done using reported disc diffusion method on common gram positive and gram negative bacteria whereas DPPH (2,2-Diphenyl-1-Picrylhydrazyl) and Nitrous oxide method was used to evaluate antioxidant activities of extract. The MEFMD showed the presence of phenolic compound, flavonoids and sterol whereas AEFMD showed the presence of flavonoids, glycosides(?), alkaloids and amino acids. Total phenolic content was expressed as GAE (Gallic acid equivalent) in mg per gram dry weight of sample. MEFMD showed more promising antimicrobial and antioxidant activity as compared to AEFMD.

Keywords: *Momordica dioica Roxb*, Antibacterial, Antioxidant.

INTRODUCTION

Momordica dioica Roxb is a perennial climbing creeper belonging to the family Curcurbitaceae and generally found in the forests of Southern India, Bengal, Maharashtra and Madhya Pradesh. In Maharashtra, the local name for fruit is *"Kartule"* and available in the month of April-July. The various parts of the plant are used for different ailments like fever, urinary complaints, bleeding piles and bowl infections. The fruit is used as alexiteric, stomachic, laxative; cure asthma, leprosy, tumors, bronchitis, troubles of heart and jaundice (1).

Hepatoprotective activity of fruits of *Momordica dioica* (FMD) has already been reported with methanolic extract. Previous study also revealed the presence of flavonoids, phenolic compound, ascorbic acid, iodine and pleuchiol (sterol).

Momordica dioica Roxb. (Ex. willd) is dioecious, perennial climbing creeper belonging to the family cucurbitaceae. It is generally found in the forests of Southern India, Bengal, Madhya Pradesh and Maharashtra.

The various parts of *Momordica dioica* (MD) are used for different ailments. The fruit is pungent, bitter, hot, alexiteric, stomachic, laxative; cures '*vata*', biliousness, asthma, leprosy, bronchitis, fever, tumors, urinary discharges, excessive salivation and troubles of heart. The fruit is rich in ascorbic acid and contain iodine (2). The fruit also contain alkaloid, flavonoids, glycosides and amino acids (3). In the present study antimicrobial and antioxidant properties of MEFMD and AEFMD were established.

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MATERIAL AND METHOD:

Chemicals:

DPPH (1, 1-diphenyl-2-picrylhydrazyl) and a-naphthylethylene diamine dihydrochloride were purchased from Sigma-Aldrich, Germany. Methanol, potassium phosphate (monobasic & dibasic), Sodium nitropruside were of research grade (Qualigens). Erythromycin standard antibiotic discs, 0.5 McFarland Standard, nutrient agar were procured from Microlabs, Mumbai.

Micro-organism:

All the authentic cultures of test microorganisms have been procured from National Collection of Industrial Microorganisms (NCIM) a division of National Chemical Laboratory (NCL), Pune. The test microorganisms were *Bacillus subtilis (ATCC 633), Bacillus cereus (ATCC 10703), Staphylococcus aureus (ATCC 9144), Escherichia coli (ATCC 8739), Pseudomonas aeruginosa (ATCC 19429) and Klebsiella pneumoniae (NCIM 2957).*

Collection and authentication:

The whole plant of MD has been collected from the forest region of Khopoli nearby Lonavala, Dist-Pune (MS) and authenticated by Botanical Survey of India, Pune. Authentication Certificate No. is BSI / WC /TECH /341 and Voucher specimen (No. MD-2008/01) has been submitted to pharmacognosy department of Sinhgad Institute of Pharmaceutical Sciences, Kusgaon (Bk), Lonavala, Pune (MS).

Preparation of extract:

Methanolic extract: 50gm of fresh MD fruits were extracted with 500ml of pure methanol on Soxhlet apparatus. The extract obtained was concentrated on rotary vacuum evaporator to get green semisolid residue (Yield 18.2% w/w).

Aqueous extract: 50gm of fresh MD fruits were extracted with 500ml of double distilled water by maceration. The extract obtained was con-

Table 1: Phytochemicals in AEFMD and MEFMD

Phyto-Constituent	AEFMD	MEFMD
Carbohydrate	-	-
Phenolic compounds	+	++
Flavonoids	+	+
Alkaloids	+	-
Glycosides	+	-
Tannins	-	-
Saponin	-	-
Steroids	-	-

++ Strong positive, + positive test, - negative test

Table 2: Antibacterial activity results of AEFMD and MEFMD.

Bacteria	MIC (mg/ml)	
	AEFMD	MEFMD
S. aureus (ATCC 9144)	12.5	12.5
B. cereus (ATCC 10703)	12.5	12.5
B. subtilis (ATCC 633)	25	12.5
K. pneumoniae (NCIM 2957)	25	12.5
E. coli (ATCC 8739)	50	25
P. aeruginosa (ATCC 19429)	50	12.5

Table 3: Antibacterial activity results of AEFMD and MEFMD (Inhibition Zone Diameter in mm)

Bacteria	50mg/disc		15 µg
	AEFMD	MEFMD	ERTCN
	Inhibition Zone Diameter in mm		
S. aureus (ATCC 9144)	12	15	23
B. cereus (ATCC 10703)	16	20	26
B. subtilis (ATCC 633)	13	18	28
K. pneumoniae (NCIM 2957)	11	16	26
E. coli (ATCC 8739)	18	30	35
P. aeruginosa (ATCC 19429)	20	29	29

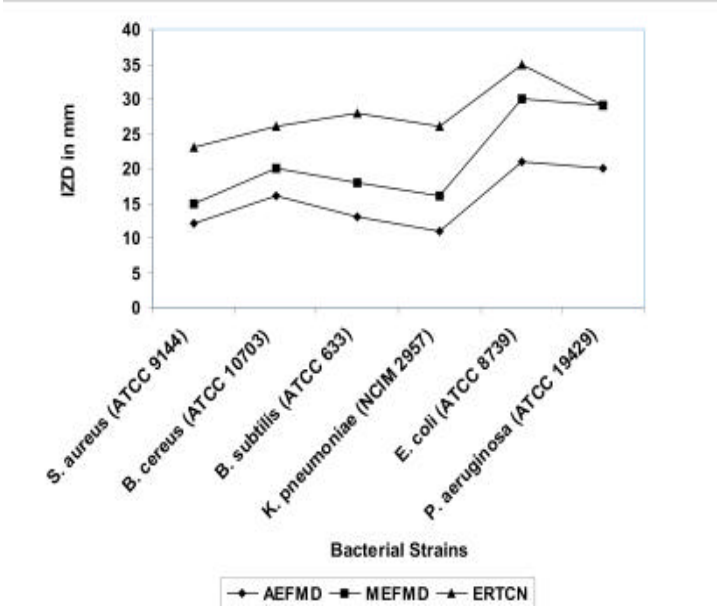

Fig. 1: Antibacterial activity results of AEFMD and MEFMD (Inhibition Zone Diameter in mm)

centrated on rotary vacuum evaporator to get green semisolid residue (Yield 22.1% w/w).

Qualitative phytochemical analysis:

MEFMD and AEFMD were screened for the presence of carbohydrate, tannins, phenolic compounds, alkaloids, anthraquinones, cyanogenetic glycosides, saponin glycosides, and steroidal nucleus

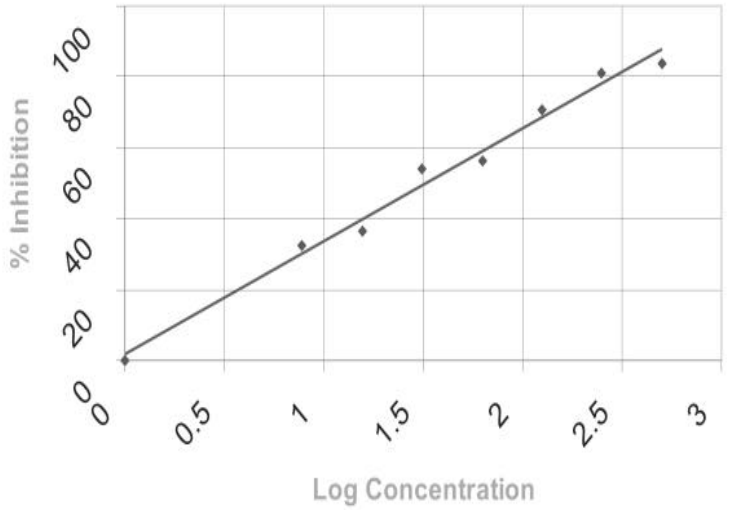

Fig. 2: Antioxidant activity results of MEFMD by DPPH.

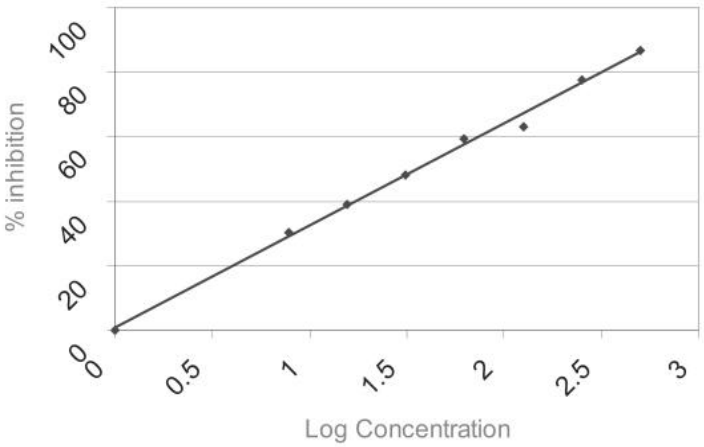

Fig. 3: Antioxidant activity results of MEFMD by Nitrous Oxide method.

using the reported methods (4-6).

Determination of total phenolic content:

The total phenolic content was determined using Folin-Ciocalteu phenol reagent and determining the absorbance at 765nm according to the reported method (7). The 200µL of diluted sample were added to 1ml of 1:10 diluted Folin-ciocalteu reagent. After 4 min, 800µL of saturated sodium carbonate solution (75g/L) was added. After incubation at room temperature, the absorbance was measured at 765 nm in triplicate. Gallic acid (0-500mg/L) was used for calibration of standard curve. The results were expressed as milligram Gallic acid equivalent (mg GAE) /g dry weight of plant materials.

Determination of MIC:

The MIC was determined by micro-broth dilution method (8). The reconstituted extract was serially diluted 2-fold in Nutrient broth (Himedia) medium. Duplicate tubes of each dilution (12.5, 25, 50 and 100 mg/ml) were inoculated with the test bacterial suspension adjusted to optical density to that of 0.5 McFarland standard and tubes were incubated at 37°C for 24 h. MIC was taken as the highest dilution (least concentration) of extract showing no detectable growth.

Table 4: In-vitro Antioxidant activity results of AEFMD and MEFMD by DPPH and Nitric Oxide Method.

Log concentration	DPPH Method			Nitric Oxide Method		
	AEFMD	MEFMD	Ascorbic acid	AEFMD	MEFMD	Ascorbic acid
	% Inhibition*	% Inhibition*	% Inhibition*	% Inhibition*	% Inhibition*	% Inhibition*
0.8927	32.23±1.21	44.25±1.41	62.56±1.22	28.44±1.19	30.45±1.28	51.16±1.24
1.1936	36.7±1.61	46.12±1.35	78.24±1.42	30.56±1.24	38.8±1.69	62.2±1.28
1.4948	54±1.82	58.3±1.24	92.77±1.13	40.12±1.22	48.18±1.44	78.11±1.46
1.7958	56.52±2.12	61.11±1.44	98.82±1.24	51.24±1.52	59.21±2.08	89.46±1.54
2.0969	70.62±1.91	72.6±1.62	ND	59.4±1.23	63.24±1.34	ND
2.3979	80.9±1.23	86.2±1.81	ND	72.6±1.42	77.8±1.22	ND
2.6989	83.62±1.14	89.98±2.14	ND	80.11±2.01	86.9±1.24	ND
IC_{50} µg/ml	33.18±1.21	16.98±1.80	7.21±1.52	54.32±1.52	35.87±1.14	13.03±1.24

*N=3, * mean ± SD, ND- not determined.*

Screening of Antibacterial Activity:

The antibacterial activity of AEFMD and MEFMD was screened using the disc diffusion method (9). Inoculum was prepared with fresh cultures of bacterial strains, cultured on Nutrient agar (Hi-media) for 18 h at 37°C with physiological saline. Inoculum density of each bacterial suspension was adjusted to reach an optical comparison to that of a 0.5 McFarland standard, resulting in a suspension containing approximately 1 to 2 x 10^8 CFU/ml (10). Nutrient agar plates were inoculated by streaking the swab over the entire sterile agar surface. This procedure was repeated by streaking 2 more times, rotating the plate approximately 60° each time to ensure even distribution of the inoculum. The inoculum was allowed to dry at room temperature. The 50mg of MEFMD and AEFMD were loaded on 6-mm sterile discs. The loaded discs were impregnated on agar media and were allowed to stand at room temperature for 30min for extract to diffuse into the agar and then they were incubated at 37°C for 24 h. Erythromycin 15µg discs were served as control. Subsequently, the plates were examined for bacterial growth inhibition and IZD was measured.

In vitro antioxidant activity:

MEFMD was subjected to *in-vitro* antioxidant activity by DPPH and Nitric Oxide radical scavenging activity (11).

DPPH Method:

37.5µg/ml solution of DPPH was prepared in methanol [15mg (15000µg) DPPH was dissolved in 10 ml of methanol. The 0.75 ml of the solution was diluted to 30 ml to give the solution of 37.5µg/ml. This concentration of DPPH gave the absorbance of 0.9]. A stock solution of 1000µg/ml was prepared by dissolving 20mg of dried MEFMD in 20ml of methanol on cyclomixer. Different concentrations were prepared from stock solution. The reaction mixer was prepared by mixing 1ml MEFMD with 1ml DPPH solution. The mixer was kept in dark for 30 min and absorbance was measured at 518nm on Jasco UV-spectrophotometer (V-530 model). The reduction in absorbance in presence of extract was recorded.

Nitrous oxide radical scavenging activity:

Sodium nitropruside in aqueous solution at physiologic pH (7.4) spontaneously generates nitric oxide, which interact with oxygen to produce nitrite ions, which can be determined by use of Griess reagent. The capacity of the *momordica dioica* to scavenge nitric oxide radicals to 50% was measured in terms of IC_{50}. Sodium nitropruside (10 Mm) in phosphate buffered was mixed with different concentration of the MD dissolved in methanol and incubated at 25°C for 150min. The sample from above was allowed to react with Greiss reagent (1% sulphanilamide,5% Ortho-phosphoric acid, and 0.1% α-naphthylethylene diamine dihydrochloride). The absorbance of the resultant coloured solution formed was taken at 546 nm against control. The percentage of nitric oxide radicals scavenging activity calculated using following equation,

$$\% \text{ Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

RESULT AND DISCUSSION:

Phytochemical evaluation of AEFMD showed the presence of phenolic compounds, flavonoids, alkaloids, glycosides whereas MEFMD shows the presence of Phenolic compounds and flavonoids *Table1*. The MIC values of AEFMD and MEFMD are reported in *Table 2*. MEFMD demonstrated promising antibacterial activity against tested gram positive and gram negative bacterias. The *B. cereus, E.coli and P. aeruginosa* shows more susceptibility to MEFMD. All the bacterias showed susceptibility to the standard antibiotic discs of Erythromycin (15 µg) (*Table 3, Figure 1)*. The MEFMD is found to be potent antioxidant. The percent inhibition calculated as IC_{50} value of MEFMD is 17.97µg/ml and 35.75 µg/ml with DPPH and NO respectively (*Table 4, Figure 2 and 3*). The total phenolic content was found to be 9.25mg GAE per gram of dry sample.

The phenolic acids and flavonoids are known to be biologically active. The phenolic compounds are known to be toxic to microorganism. The site and number of hydroxyl groups on the phenol group are thought to be related to their relative toxicity to the microorganisms. The probable mechanism responsible for phenolic toxicity to microorganisms include enzyme inhibition by oxidized compounds possibly through reaction with sulfhydryl groups or through more nonspecific interaction with proteins (12). Flavonoids are also hydroxylated phenolic substances but occur as a C_6-C_3 unit linked to an aromatic ring. Since they are known to be synthesized by plants in response to microbial infection hence they have been found *in-vitro* to be effective antimicrobial substances against a wide array of microorganisms (12).

Free radicals are produced in our body as a result of oxidative biochemical metabolisms. These free radical cause damage to cells and are responsible for aging and other ailments such as cancer, Alzheimer's disease that are life threatening and mostly fatal (12). Flavonoids are flavone-like substances that are usually antioxidants. Flavonoids scavenge free radicals by forming a stable radical that can react with another flavonoid radical to produce two non-radicals.

The present investigation revels that MEFMD showed significant antimicrobial and antioxidant activity compared with known antibiotic erythromycin and antioxidant ascorbic acid respectively.

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