

Review

Phytopharmacological Evaluation of *Momordica balsamina* Linn. From Southern Haryana, India

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Abstract

Momordica balsamina Linn. (Cucurbitaceae), a tendril bearing wild climber has been used as a traditional folk medicine in many countries. It has wide spectrum of medicinal and nutritional values. The fruits of widely available *M. balsamina* from Southern Haryana were selected for the present study. The phytopharmacological evaluation was performed to prove its medicinal importance. Preliminary phytochemical analysis, quantitative evaluation of phytoconstituents and estimation of antioxidant potential, In vitro antimicrobial activity of various extracts of *M. balsamina* fruits were determined by using different methods. Preliminary phytochemical screening of fruits showed the presence of fatty acids, carbohydrates, tannins, flavonoids, sterols, saponins and alkaloids. The amount of total polyphenols and flavonoids expressed respectively as 201.14 mg/g gallic acid and 103.01 mg/g quercetin in methanol extract of fruits while in the aqueous extract, it was 102.41 mg/g gallic acid and 83.01 mg/g quercetin respectively. The antioxidant potential was studied using different models like DPPH radical scavenging activity, hydrogen peroxide scavenging assay, reducing power assay and phosphomolybdate assay. Antimicrobial activity of methanol and aqueous extracts of fruit was determined by cylinder plate assay method by comparing inhibition zones produced by different microbes viz. *Pseudomonas aeruginosa* (MTCC 424), *Enterococcus faecalis* (MTCC 2729), *Klebsiella pneumoniae* (MTCC 432), *Staphylococcus aureus* (MTCC 3160), *Candida albicans* (MTCC 227) and *Aspergillus niger* (NCIM 501). In this paper we are reporting the preliminary phytoconstituents screening, quantitative estimation of phytoconstituents, antioxidant and antimicrobial activity of *M. balsamina* fruits.

Key words: *Momordica balsamina* Linn; Fruit; Cucurbitaceae; Antioxidant; Antimicrobial.**Introduction**

The genus *Momordica* belongs to the tribe Joliffieae (Schrad.), of the family Cucurbitaceae, distributed in warm tropics of both hemispheres, native to the tropical regions of Africa, Asia, Arabia, India and Australia [1-3]. In India *M. balsamina* is restricted to the arid belt [4,5]. *M. balsamina* (Balsam apple,

African pumpkin, bitter cucumber or bitter melon) is a high-climbing annual to perennial tendril-bearing herb. This plant is a monoecious climber, orange to red spindle-shaped ripe fruit and found in India up to an altitude of 300 m, occurs naturally in forest, in the rainy season [1-3, 6, 7]. It is a wonder plant for nutraceutical sciences. The leaves are important source of nutrients having 17 amino acids [8]. The leaves, fruits, seeds,

and bark are reported to have various medicinal and nutritional importance and called 'Hidden gift of Nature'[7-11]. Young peeled fruits are cooked and eaten; they are often steeped in salt water after peeling and before cooking to remove its bitter taste [12]. The fruits are common ingredients in Indo-Pakistan pickles and are often used in curries and meat dishes. The leaves and fruits were observed to have hypoglycemic effects [13, 14]. The fruits are occasionally used in native practice. It is famous in Nigeria and in Syria for curing wounds, as hemostatic antiseptic [7,15]. Fruits pulps extract of this plant has given valuable information on anti-HIV property [16]. Fruit extracts of this plant show, antiplasmodial activity and is being used against malaria in african traditional medicine [17-18]. Despite the rich pharmacological potential of fruits of the plant, not much research work has been done to scientifically evaluate it, thus the present research work was conducted to study preliminary phytoconstituents, antioxidant and antimicrobial activity of *M. balsamina* Linn. fruits.

Material and Methods

Chemicals and reagents

All the chemicals and reagents used were of analytical grade.

Plant materials

Momordica balsamina Linn fruits were collected in Southern region of Haryana, India in June 2015. The plant was taxonomically identified and authenticated by Dr. Anjula Pandey, Principal Scientist Raw Materials, Herbarium and Museum Division, NISCAIR, New Delhi, vide reference number NHCP/NBPGR/2016-13(*Momordica balsamina* Linn) dated 17 March, 2016. A voucher specimen of the same has been retained in the Department for the future reference. The fruits were dried under shade and coarsely powdered for further study.

Preparation of plant extracts

The powdered plant fruits were extracted with petroleum ether, chloroform and methanol. The extraction was done by hot

continuous soxhlet extraction method. The marc obtained after methanol extraction were air dried and cold maceration was done with distilled water to get aqueous extract. The extracts were concentrated by rotary vacuum evaporator and lyophilized. The extracts were kept in desiccator till further use. The percentage yield of petroleum ether, chloroform, methanol and aqueous extracts were 4.2%, 5%, 17%, 15% w/w respectively. The extracts were further used to perform preliminary phytochemical screening, quantitative estimation of phytoconstituents, In vitro antioxidant and antimicrobial activities.

Preliminary phytochemical study

The petroleum ether, chloroform, methanol and aqueous extracts of fruits were subjected to preliminary phytochemical screening using standard method of analysis [19-22]. They were screened for the presence of alkaloids, carbohydrates, glycosides, saponins, proteins and amino acids, phenolic, sterols, flavonoids and tannins.

Test for Alkaloids

About 500 mg of each of the dried extract was stirred with about 5 mL of dilute hydrochloric acid and filtered. The filtrate was tested with the following reagents:

Mayer's Reagent: Few drops of Mayer's reagent (Potassium mercuric iodide solution) were added separately to each filtrate and observed for the formation of white or cream coloured precipitate.

Dragendroff's Reagent: Few drops of Dragendroff's reagent (solution of potassium bismuth iodide) were added separately to each filtrate and observed for the formation of reddish brown colour precipitate.

Hager's Reagent: Few drops of Hager's reagent (saturated aqueous solution of picric acid) were added separately to each filtrate and observed for the formation of yellow precipitate.

Test for Glycosides

Killer-Killani test: 1 mL of glacial acetic acid containing traces of ferric chloride and 1 mL of concentrated sulphuric

acid was added to extracts carefully. Appearance of red colour indicates the presence of glycosides.

Sodium Nitroprusside test: The extracts were made alkaline with few drops of 10% sodium hydroxide and then freshly prepared sodium nitroprusside solution was added. Blue colour indicates the presence of glycosides in the extracts.

Borntrager test: Appearance of pink colour, when 1 mL of benzene and 0.5 mL of dilute ammonia solution were added to extracts indicates positive test for glycosides.

Test for Carbohydrates

Molish test: A few milligram of extract was dissolved in water and filtered. To the filtrate few drops of α -naphthol (20% in ethyl alcohol) were added. Then about 1 mL of concentrated sulphuric acid was added along the side of the tube, reddish violet ring at the junction of two layers was seen, indicates the presence of carbohydrates.

Fehling's test: 1 mL of Fehling's reagent (Copper sulphate in alkaline conditions) was added to filtrate of extract in distilled water and heated on a steam bath. Appearance of brick red precipitates indicates the presence of carbohydrates (as reducing sugars).

Test for Saponins

Foam test: To the few milligrams of the extract, few drops of water were added and shaken well. Formation of foam indicates the presence of saponins.

Sodium bicarbonate test: To the few milligrams of extract, few drops of sodium bicarbonate were added and shaken well. Formation of honey comb like frothing indicates positive test for saponins.

Test for Proteins and Free Amino Acids

Few milligram of residue, 5 mL distilled water was added and filtered. Filtrate was then subjected to the following tests:

Biuret test: To the ammoniated alkaline filtrate 2-3 drops of 0.02% copper sulphate solution were added. Appearance of a

red/violet colour indicates the presence of proteins and free amino acids.

Ninhydrin test: To the filtrate, lead acetate solution was added to precipitate tannins and filtered. The filtrate was spotted on a paper chromatogram, sprayed with ninhydrin reagent and dried at 110°C for 5 minutes. Violet spots (free amino acids) confirmed the presence of proteins/free amino acids.

Test for phenolic compounds

A few milligram of extract was mixed with 5 mL of distilled water, filtered and to the filtrate following tests were performed.

Test for tannins

Ferric Chloride test: Formation of blue - black colour on addition of ferric chloride solution (5%) was taken as positive test for tannins (gallic tannins).

Lead Acetate test: Addition of few drops of lead acetate solution (5%) to the aqueous extract gives precipitate, suggesting the existence of tannins.

Test for Flavonoids

Ammonia test: Filter paper strip was dipped in the filtrate, and ammoniated. The filter paper strip turned yellow indicates the presence of flavonoids.

Shinoda test: A piece of metallic magnesium was added to the filtrate, followed by addition of 2 drops of concentrated hydrochloric acid. Appearance of reddish brown colour indicates the presence of flavonoids in all the extracts.

Test for Sterols

Liebermann-Burchard test: A few milligram of extract was dissolved in chloroform and few drops of acetic anhydride was added along with a few drops of concentrated sulphuric acid from the sides of the tube. The appearance of blue to blood red colour indicates sterols in the extract.

Salkowski reaction: 2 mL of concentrated sulphuric acid was added to few milligram of residue extract. The appearance of a yellow ring at the junction which turns red after 1 min indicates the presence of sterols in the extract.

Quantitative Estimation of Phytoconstituents

Total alkaloids estimation

Each extract (50 mg) was mixed with 200 mL of acetic acid (10%) in ethanol; the beaker was covered and incubated for 4 h. The mixture was concentrated up to one third of its total volume. Ammonium hydroxide was added drop wise in the mixture until it formed precipitate. The precipitate was washed with ammonium hydroxide and then filtered. The filtrate (alkaloids) was calculated as percentage of the dried fraction [19].

Total carbohydrates

100mg of galactose were dissolved in 100 mL distilled water. Then 10 mL of strong galactose solution was dissolved in 100 mL distilled water to make the dilute galactose solution. Dilute galactose, mannose and sample were read [23].

Carbohydrates as galactose (or mannose) % = $25 \times B/S \times A$

Where: B = reading of sample, A = reading of dilute galactose (or mannose) and S = weight of origin sample.

Total saponin estimation

Methanol extract and each fraction (50 mg) were mixed in 100 mL of ethanol (20%). It was kept on heating for 4 h with continuous stirring at 55°C, than diluted with diethyl ether (20 mL) and washed with 5% sodium chloride. Saponins were estimated as percentage of the dried fraction [19].

Determination of Fat Content

Accurately weighted an extraction flask containing a few glass beads and then added approximately 250 mL of petroleum ether in it. Extracted the sample (5g) contained in the thimble for at least 80 cycles in a minimum of 4 hours in a Soxhlet extraction apparatus. Upon completion of the extraction,

separated the unit and poured off the ether (and thimble) from the extractor into a large filter (to collect the thimbles) positioned on a container. Repeated until most of the ether was removed and the flask had very little ether left. Took apart the Soxhlet unit and placed flask on a steam bath to evaporate the remaining petroleum ether. Swirled flask initially to avoid boil-over. Dried flask and its contents in a mechanical convection oven at 100 - 102 °C for time required to obtain constant weight. Cooled to room temperature [23].

$$(\%) \text{ Fat content} = 100(B - C)/A$$

Where A = Sample weight; B = Weight of flask after extraction; C = Weight of flask prior to extraction

Total phenolic content

The total phenolic content was estimated by Folin-Ciocalteu reagent as earlier reported method. Gallic acid stock solution (1000µg/mL) was prepared. Various dilutions of standard gallic acid were prepared from this stock solution. Calibration curve (Figure 1) was plotted by mixing 1mL aliquots of 5, 10, 25, 50 and 75 µg/mL of gallic acid solutions with 5 mL of Folin-Ciocalteu reagent (diluted ten time) and 4 mL of sodium carbonate solution (75g/l). The absorbance was measured after 30 minutes at 765 nm [24,25].

$$\text{Total phenolic compound PC} = C_g \times V/M$$

Where; PC=total content of phenolic compounds in mg/g, in GAE (gallic acid equivalent); C_g= the concentration of Gallic acid established from the calibration curve in mg/mL; V=the volume of extract in mL; M=the weight of plant extract in gm. All the tests and analysis were run in triplicates and averaged.

The total flavonoid content

The total flavonoids content of various extracts was determined using a colorimetric method. A volume of 0.5mL of 2% aluminium chloride ethanol solution was added to 0.5mL of samples. After one hour at room temperature, the absorbance was measured at 420nm. A yellow color indicated the presence of flavonoids. Extract samples were evaluate at a final concentration of 0.1mg/mL [24,26-28]. Total flavonoid

content was calculated as quercetin equivalent (mg/g) using a standard curve with quercetin (0 - 100 µg/mL) as the standard and calculated using the formula: $FC = Cq \times V/M$

Where; FC=total flavonoid content in mg/g, in quercetin equivalent; Cq = the concentration of quercetin established from the calibration curve in mg/mL; V=the volume of extract in mL; M=the weight of plant extract in g. All the tests and analysis were run in triplicates and averaged.

***In-vitro* antioxidant assays**

A stock solution (1mg/mL) of the extract was prepared in methanol and diluted for various antioxidant assays. Antioxidant power of each assay was compared with the efficacy of standard chemicals.

DPPH radical scavenging activity

0.02-0.1mg of the extract in methanol was mixed with 1 mL of 0.135 mM DPPH in methanol solution and 450 µl of 50 mM Tris- HCl buffer (pH 7.4). Methanol (50 µl) was used as the experimental control [20, 29]. After 30 min of incubation at room temperature, the reduction of DPPH free radicals was measured, read the absorbance at 517 nm Ascorbic acid was used as standard. The percent inhibition was calculated from the following equation:

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

Hydrogen peroxide scavenging

Hydrogen peroxide (H₂O₂) enters the human body through inhalation of vapor or mist and through eye or skin contact. In the body, H₂O₂ is rapidly decomposed into oxygen and water and this may produce hydroxyl radicals (OH[·]) that can initiate lipid peroxidation and cause DNA damage. The ability of plant extracts to scavenge hydrogen peroxide is determined .A solution of hydrogen peroxide (20 mM) was prepared in phosphate buffer saline (pH 7.4). 1mL of various concentrations of plant extracts and standard ascorbic acid solution (10, 20, 40, 60, 80 and 100 µg/mL in methanol) was

added to 2 mL of hydrogen peroxide solution. Absorbance of hydrogen peroxide at 230nm was determined after 10 minutes against a blank solution containing phosphate buffer without hydrogen peroxide [20,30-32]. The percentage inhibition was calculated using the following formula:

$$\text{Percentage inhibition} = [(A_0 - A_t/A_0) \times 100]$$

Where A₀ is the absorbance of the control and A_t is the absorbance of test extract. All the tests and analysis were run in triplicates and averaged.

Reducing power method (RP)

This method is based on the principle of increase in the absorbance which indicates an increase in the antioxidant activity. In the method 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of potassium ferricyanide (1% w/v) was added to 1.0 mL of different extracts solution. The resulting mixture was incubated at 50°C for 20 min and added 2.5 mL of trichloroacetic acid (10% w/v). The mixture was centrifuged at 3000 rpm for 30 min. Collected the upper layer of the solution (2.5 mL) and mixed with distilled water (2.5 mL) and 0.5 mL of ferric chloride (0.1%, w/v). The absorbance is then measured at 700 nm against blank. Ascorbic acid was used as standard and phosphate buffer as blank solution [32].

Ferrous chelating ability

The chelation of ferrous ions was estimated using, 1.0 mL of different extracts solution in the concentration range of 10-100 µg/mL separately were added to a solution of 0.1 mL ferrous chloride (2 mM) and 3.7 mL of methanol. The reaction was initiated by the addition of 0.2 mL of ferrozine (5 mM) and incubated at room temperature for 10 min and then the absorbance was measured at 562 nm. The IC₅₀ values were compared with ascorbic acid [33-35].The percentage inhibition of ferrozine- Fe²⁺ complex formation was calculated as:

$$[(A_0 - A_s)/A_s] \times 100$$

Where A₀ was the absorbance of the control, and A_s was the absorbance of the extract or ascorbic acid (positive control).

Total antioxidant capacity (Phosphomolybdate assay)

To different extracts, 0.1mL (100 µg/mL) solution was combined with 1.0 mL of reagent (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) and tubes were incubated in a boiling water bath at 95°C for 90 min. Cooled the solution at room temperature and the absorbance was measured at 695 nm. The blank solution contained 1 mL of reagent solution and the appropriate volume of the same solvent used for the sample and it was incubated under same conditions as rest of the sample [35].

Antimicrobial Study

A total of four bacterial and two fungal species were used in this study. Microorganisms were procured from Microbial type culture collection (MTCC), Chandigarh, National collection of industrial microorganism (NCIM), Pune [36].

Strains for antibacterial activity

Pseudomonas aeruginosa (MTCC 424), *Enterococcus faecalis* (MTCC 2729), *Klebsiella pneumoniae* (MTCC 432) and *Staphylococcus aureus* (MTCC 3160),

Strains for antifungal activity

Candida albicans (MTCC 227) and *Aspergillus niger* (NCIM 501).

Culture Media

The culture media used for antimicrobial assay were procured from HiMedia Bombay, India. Media were prepared using specified quantities for antibiotic assay medium and were there after sterilized by autoclaving at 15lb/square pressure at 121°C for 20 minutes [37].

Media for bacterial growth

i) Nutrient Broth Medium

Beef extract 10 g, Peptone 10 g, Sodium chloride 5 g, Distilled water 1000 mL, pH adjusted to 7.0

ii) Nutrient Agar Medium

Beef extract 10 g, Peptone 10 g, Sodium chloride 5 g, Agar 20 g, Distilled water 1000 mL, pH adjusted to 7.0

Media for fungal growth

i) Sabouraud's Dextrose Agar Medium

Dextrose 40 g, Peptone 10 g, Agar 15 g, Distilled water 1000 mL, pH adjusted to 5.6

ii) Sabouraud's Dextrose Broth Medium

Dextrose 40 g, Peptone 10 g, Distilled water 1000 mL, pH adjusted to 5.6

Experimental Work

Preparation of Test Inoculums

(a) Seeded broth preparation: The stock bacterial cultures were maintained in nutrient agar slants at 4°C. Each of the microorganisms was freshly cultured prior to susceptibility testing by transferring them into a separate sterile conical flask containing about 100 mL nutrient broth and incubated overnight at 37°C and termed as seeded broth. A microbial loop was used to remove a colony of each bacteria from pure culture and transferred into nutrient broth[38].

(b) Standardization of seeded broth (viable count)

(i) Dilution: In 99 mL of sterile water containing 0.05% Tween 80, 1 mL of seeded broth was added. From this, 1 mL was taken and diluted to 10 mL with sterile water and seeded broth is further diluted upto 10-10 dilution.

(ii) Inoculation into nutrient agar petri dishes 0.2 mL of seeded broth dilutions were inoculated into solidified nutrient agar medium by spread plate method. Number of colonies of microorganisms formed after inoculation at 37°C. The seeded broth was suitably diluted to contain 10⁶-10⁷ colony forming unit/mL (cfu/mL). It was the working stock and used for microbiological evaluation.

Zone of Inhibition

Cylinder-plate method or Cup-plate method

This method depends upon the diffusion of an antibiotic from vertical cylinder or a cavity through the solidified agar layer of a petridish or plate to an extent such that growth of the added microorganisms is prevented entirely in a circular area of zone around the cylinder or cavity containing the solution of test compound. Diameter of the clear zone produced due to inhibition of microbial growth is measured.

Preparation of stock and standard solutions

The solution of test compounds and standards were prepared at the concentration of 1000 µg/mL by dissolving them with distilled water in small volumetric flasks [36, 39].

Procedure

The standard and test compounds (methanol and aqueous extract of plant) solution were prepared in DMSO at the concentration of 10 mg/mL. Standard drugs used in the study were Ofloxacin at the concentration of 1 mg/mL for bacterial assay and Clotrimazole (1 mg/mL) for the assay of fungi. The petriplates containing 25 mL of sterile nutrient agar were inoculated with standardized inocula (0.1×10^8 cell/mL) using sterile Pasteur pipette. Wells of 8mm diameter were made by steel borer at the centre of each plate. To these wells 0.2 mL of various test and standard compounds solution were dispensed aseptically into each well. The extracts were allowed to diffuse into medium for 1 hour at room temperature. The plates were incubated at 37°C for 18 hours for bacteria and 37°C for 72 hours for *C.albicans* and 28°C for *A. niger* for a period of seven days. Antimicrobial potential of test compound was determined on the basis of mean diameter of zone of inhibition around the wells. The experiment was repeated thrice and the average values were recorded. As appreciable results in form of significant zone of inhibition was seen so minimum inhibitory concentration of various test compounds was also screened.

Minimum Inhibitory Concentration (MIC)

MIC of extracts was determined using turbidity method in nutrient broth medium for bacterial strains and Sabouraud dextrose broth medium for fungal strains. This method depends upon the growth of a microbial culture in uniform solution of the test solution in a fluid medium that is favorable to its rapid growth in the absence of test compound. Varying concentration of the compounds was added to test organism on liquid culture [39].

Result and Discussion

Preliminary Phytochemical Screening

The preliminary phytochemical screening was found that alkaloids, glycosides, carbohydrates, saponins, proteins, aminoacids, flavonoids, sterols, phenolic compounds and tannins were presents in different extracts of plant (Table 1).

Total phenolic content

The total phenolic content was determined using Folin-Ciocalteu method. The results are reported as gallic acid equivalents by reference to standard curve ($y = 0.010x + 0.044$ and $r^2 = 0.998$, where y was the absorbance and x is concentration of gallic acid in µg/mL) as shown in figure 1. The results showed that total phenolic content was more in methanol extract of fruits than other test extracts of fruits (Table 3).

Total flavonoids content

The total flavonoids content of various extracts was calculated (mg/g) using the standard curve of quercetin from equation ($y = 0.010x + 0.044$, $R^2 = 0.998$, where y was the absorbance and x is concentration of quercetin in µg/mL) as shown in figure 2. The total flavonoid content in various extracts of fruits is shown in Table 3. In fruits the total flavonoids content was found to be more in methanol extract followed by aqueous, petroleum ether and chloroform extract.

In case of *M. balsamina* Linn. fruits free radical effect of methanol extract was more than the aqueous extract

followed by chloroform extract. The activity of the methanol extract of *M. balsamina* Linn. fruits was found to be comparable with ascorbic acid with IC₅₀ values of 39.03±0.2, 44.03±0.5 and 10±0.5 µg/mL respectively.

Hydrogen Peroxide scavenging activity

Hydroxyl radicals scavenging activity was quantified by measuring inhibition of the degradation of the deoxyribose by free radicals. Studies have attributed that antioxidant properties are due to the presence of phenols and flavonoids. The hydrogen peroxide scavenging activity of petroleum ether, chloroform, methanol and aqueous extracts of *M. balsamina* Linn. fruit are depicted in Table 5 and Figure 4.

In *M. balsamina* Linn. fruit methanol and aqueous extract displayed strong scavenging activity as compared to the chloroform and petroleum ether extract. In fruit, the activity of the methanol extract was found to be comparable with ascorbic acid with IC₅₀ values of 30.02±0.30µg/mL, and 21.51±0.10µg/mL respectively.

Reducing power assay

Reducing power assay of plant extracts and EDTA (Positive control) was observed a concentration range of 10-100 µg/mL and the results are shown in Tables: 6 and Figure 5.

The reducing power assay of all the extracts was found to be increasing with increasing concentration. It was found methanol extract was found with IC₅₀ of 35.2 µg/mL and IC₅₀ of EDTA which was found to be 20.03 µg/mL. Methanol extracts of *M. balsamina* Linn. fruit showed better anti-oxidant activity in the test-model than other extracts.

The different extracts were monitored by measuring the formation of the ferrous ion ferrozine complex. It was found methanol extract was found with IC₅₀ 48.2 µg/mL and IC₅₀

of Ascorbic acid which was found to be 30.11 µg/mL. Methanol extracts of *M. balsamina* Linn. fruit showed better result than other extracts.

Total antioxidant activity (phosphomolybdate method)

The total antioxidant capacity of the different extracts was determined by phosphomolybdate method using α tocopherol as the standard.

The research findings revealed the presence of alkaloids, glycosides, carbohydrates, saponins, phenolic, sterols, flavonoids, protein and free amino acids in the fruit extracts. The methanol extract showed more amount of phytoconstituent than other test extracts viz. carbohydrates (18.00±0.32%), saponins (18.00±0.12 µg/mL), alkaloids (5.20±0.23 µg/mL), Nitrogen (28.05 IU), fat content(14%), total phenolic content were (201.14±0.20 mg/g), total flavonoid content (103.01±1.10 mg/g). The secondary metabolites are responsible for various type of pharmacological activities. Though all the test extract exhibited antioxidant activity but methanol extract showed better results than other tested extracts. The fruits methanol and aqueous extracts when tested for antimicrobial activities in terms of zone of inhibition, methanol extract showed better zone of inhibition than aqueous extract in both antibacterial and antifungal studies. The results were compared with standard drugs. Thus the present study would help to encourage the relevance to cultivate this wild plant which is having lots of medicinal potential.

PLANT CONSTITUTENTS	TEST	FRUIT			
		PE	C	M	A
Alkaloids	1.Mayer 'reagent	+	+	++	++
	2.Dragendroff' reagent	-	-	++	++
	3.Hager'reagents	-	-	++	++
Glycosides	1.Baljet test	+	+	+	+
	2.Borntrager test	+	+	+	+
	3.Legal test	+	+	+	+
Carbohydrates	1.Molish's reagent	+	-	++	++
	2.Fehling solution	+	-	++	++
	3. Benedict test	+	-	++	++
Saponins	1.Foam test	-	+	++	++
	2.Sodium bicarbonate	-	+	++	++
Tannins	1.Ferric chloride test	+	+	++	++
	2.Lead acetate test	+	+	++	++
Flavonoids	1.Ammonia test	-	+	++	++
	2.Shinoda test	-	+	++	++
Proteins & Aminoacids	1.Biuret test	+	-	++	-
	2.Ninhydrin reagent	+	-	++	-
Sterols	1.Liebermann- Burchard test	+	+	++	+
	2. Salkowski reaction	+	+	++	+

+ve: Traces,++ve: good yield -ve: absent; PE- petroleum ether; C- chloroform; M- methanol; A- aqueous extract

Table 1 : Phytochemical screening of extracts of *M. balsamina* Linn. Fruits

Quantitative Estimation of Phytoconstituents

S.No	Tests	Fruit methanol extract	Fruit aqueous extract
1	Carbohydrate (%)	18±0.32	14.2±0.16
2	Saponin (µg/mL)	18±0.12	15.1±0.34
3	Alkaloids (µg/mL)	5.2±0.23	4.0±0.26
4	Fat content (%)	14	10

All values are expressed as mean±SEM (n=3)

Table 2: Test result of Carbohydrate, saponin, alkaloid and nitrogen and fat contents

Extract	Total Phenolic content (mg/g)	Total Flavonoid content (mg/g)
PEF	10.4±0.4	7.5±1.7
CEF	2.7±0.1	4.7±1.0
MEF	201.14±0.2	103.01±1.1
AEF	102.41±0.3	83.01±1.2

PEF: petroleum ether extract fruits CEF: chloroform extract fruits, MEF: methanol extract fruits, AEF: aqueous extract fruits.
Values are expressed as mean±SD (n=3).

Table 3: Total phenolic and flavonoid content of extracts of *M. balsamina* Linn. Fruits

Conc (µg/mL)	Percentage Inhibition(±S.E.M)*				
	Ascorbic Acid	Pet ether extract	Chloroform extract	Methanol extract	Aqueous Extract
10	50.07±0.9	18.34±0.3	22.11±0.1	39.31±0.2*	35.10±0.1*
20	55.51±0.7	25.05±0.4	30.12±0.2	44.24±0.4**	40.41±0.3*
40	63.21±0.7	31.90±0.6	37.18±0.1	52.41±0.4**	48.01±0.4*
60	71.52±0.2	38.21±0.1	44.21±0.7	59.73±0.3**	56.70±0.5**
80	82.22±0.8	43.02±0.4*	49.14±0.2*	66.37±0.2***	63.02±0.2**
100	91.71±0.3	55.25±0.4*	58.31±0.5*	75.19±0.2***	69.21±0.7**
IC ₅₀	10±0.5	91.12±0.1*	82.01±0.4*	39.03±0.2***	44.03±0.2**

All values are expressed as mean±SEM (n=3), The extracts groups are compared by Student t test with the ascorbic acid, *** P < 0.001, ** P < 0.01, *P < 0.05.

Table 4: Effect of *M. balsamina* Linn. fruit extracts on DPPH Radical Scavenging activity

Concentration (µg/mL)	Percentage Inhibition(±S.E.M)*				
	Ascorbic acid	Pet ether extract	Chloroform Extract	Methanol extract	Aqueous extract
10	42.25±0.2	22.1±0.3	28.32±0.3	40.41±0.2	35.17±0.3
20	49.4±0.1	28.34±0.2	34.45±0.4	45.37±0.6	40.51±0.2
40	58.53±0.4	37.61±0.3	43.54±0.1	55.92±0.3*	50.91±0.5
60	69.83±0.5	47.31±0.7	54.35±0.2*	64.74±0.2**	59.35±0.3*
80	77.35±0.2	54.02±0.8	62.43±0.3**	72.31±0.5**	67.36±0.2**
100	87.34±0.2	64.22±0.2	69.48±0.6**	79.42±0.3**	74.93±0.6**
IC ₅₀	21.51±0.1***	70.25±0.4	50.03±0.2**	30.02±0.3***	40.03±0.2***

All values are expressed as mean±SEM (n=3), The extracts groups are compared by Student t test with the ascorbic acid, *** P < 0.001, ** P < 0.01, *P < 0.05.

Table 5: Effect of *M. balsamina* Linn. fruit extracts on Hydrogen Peroxide Scavenging activity

Conc ($\mu\text{g/mL}$)	Percentage Inhibition($\pm\text{S.E.M}$) [*]				
	EDTA	Pet ether	Chloroform	Methanol	Aqueous
10	42.11 \pm 0.2	15.32 \pm 0.13	20.47 \pm 0.1	34.91 \pm 0.1***	25.04 \pm 0.3**
20	50.71 \pm 0.31	21.45 \pm 0.02	26.41 \pm 0.3	40.82 \pm 0.4***	31.46 \pm 0.4**
40	63.4 \pm 0.1	32.51 \pm 0.1	38.82 \pm 0.2	53.04 \pm 0.3**	42.75 \pm 0.2*
60	76.9 \pm 0.12	44.34 \pm 0.2	51.41 \pm 0.3	65.92 \pm 0.3**	56.73 \pm 0.1*
80	88.62 \pm 0.16	56.2 \pm 0.2	64.04 \pm 0.21	78.41 \pm 0.12**	70.75 \pm 0.2*
100	99.25 \pm 0.3	69.31 \pm 0.3	76.8 \pm 0.5*	92.03 \pm 0.1**	82.35 \pm 0.3*
IC ₅₀	20.03 \pm 0.3	70.03 \pm 0.5	58.4 \pm 1.3**	35.2 \pm 1.3**	49.03 \pm 0.2*

All values are expressed as mean \pm SEM (n=3), The extracts groups are compared by Student t test with the EDTA, *** P < 0.001, ** P < 0.01, *P < 0.05.

Table 6: Effect of *Momordica balsamina* Linn. fruit extracts on Reducing power assay

Concentration ($\mu\text{g/mL}$)	Ferrous chelating ability Percentage Inhibition($\pm\text{S.E.M}$) [*]				
	Ascorbic acid	Pet ether extract	Chloroform Extract	Methanol extract	Aqueous extract
10	35.51 \pm 0.1	12.41 \pm 0.2	08.22 \pm 0.2	31.02 \pm 0.7	24.23 \pm 0.6
20	45.03 \pm 0.3*	20.33 \pm 0.3	14.51 \pm 0.4	38.22 \pm 0.2	30.31 \pm 0.1
40	55.32 \pm 0.1**	29.40 \pm 0.1	24.23 \pm 0.1	47.03 \pm 0.1	39.42 \pm 0.2
60	66.32 \pm 0.3**	38.21 \pm 0.3	34.43 \pm 0.5	56.93 \pm 0.1	49.11 \pm 0.3
80	78.21 \pm 0.4**	49.04 \pm 0.6	42.51 \pm 0.2	68.11 \pm 0.4*	58.42 \pm 0.4*
100	87.34 \pm 0.2***	59.46 \pm 0.1	50.14 \pm 0.7	79.20 \pm 0.5**	69.63 \pm 0.2**
IC ₅₀	30.11 \pm 0.5***	81.55 \pm 0.5	100.03 \pm 0.8	48.31 \pm 0.3***	61.03 \pm 0.5**

All values are expressed as mean \pm SEM (n=3), The extracts groups are compared by Student t test with the standard (Ascorbic acid) *** P < 0.001, ** P < 0.01, *P < 0.05.

Table 7: Effect of *M. balsamina* Linn. fruit extracts on Ferrous chelating ability

S.No	Extracts	Total antioxidant activity (μg vitamin E equivalent/ 100 μg)
1	Petroleum ether	10.04 \pm 0.03
2	Chloroform	13.02 \pm 0.01
3	Methanol	25.90 \pm 0.05
4	Aqueous	18.36 \pm 0.02

All values are expressed as mean \pm SEM (n=3), The extracts groups are compared by Student t test with the standard (Tocopherol)

Table 8: Total antioxidant activity (phosphomolybdate method) of different extracts *M. balsamina* Linn. Fruit.

Antimicrobial Activity

S.No	Bacterial strain	Zone of Inhibition in mm		
		Ofloxacin	Methanol extract	Aqueous extract
1	<i>Pseudomonas aeruginosa</i> (MTCC 424)	20.14±0.72** *	17.32±0.92**	16.43±0.33**
2	<i>Enterococcus faecalis</i> (MTCC 2729)	21.53±0.31** *	16.62±0.15**	12.65±0.03*
3	<i>Klebsiella pneumoniae</i> (MTCC 432)	22.1±0.25***	18.23±0.14**	10.59±0.23*
4	<i>Staphylococcus aureus</i> (MTCC 3160)	18.23±0.4***	13.42±0.82**	11.53±0.21*

All values are expressed as mean±SEM (n=3), The extracts groups are compared by Student t test with the ofloxacin, *** P < 0.001, ** P < 0.01, *P < 0.05.

Table 9: Percentage of the zone of inhibition for the methanol and aqueous extract of *M. balsamina* Linn. Fruit

S.No	Bacterial strain	Minimum Inhibition Concentration (MIC) (µg/mL)		
		Ofloxacin (1mg/mL)	Methanol extract (1mg/mL)	Aqueous extract (1mg/mL)
1	<i>Pseudomonas aeruginosa</i> (MTCC 424)	50	300	350
2	<i>Enterococcus faecalis</i> (MTCC 2729)	50	300	350
3	<i>Klebsiella pneumoniae</i> (MTCC 432)	50	300	350
4	<i>Staphylococcus aureus</i> (MTCC 3160)	50	300	350

Table 10: Minimum Inhibition Concentration for the methanol and aqueous extract of *M. balsamina* Linn. Fruit

S. No	Fungal strain	Zone of Inhibition in mm		
		Clotrimazole	Methanol extract	Aqueous extract
1	<i>Candida albicans</i> (MTCC 227)	23.12±0.35***	16.22±0.46**	13.59±0.79**
2	<i>Aspergillus niger</i> (NCIM 501)	22.33±0.26***	18.24±0.52**	15.02±0.41*

All values are expressed as mean±SEM (n=3), The extracts groups are compared by Student t test with the standard (Clotrimazole) *** P < 0.001, ** P < 0.01, *P < 0.05.

Table 11: Percentage of the zone of inhibition for the methanol and aqueous extract of *M. balsamina* Linn. Fruit

S. No	Fungal strain	Minimum Inhibition Concentration (MIC) ($\mu\text{g/mL}$)		
		Clotrimazole	Methanol extract	Aqueous extract
1	<i>Candida albicans</i> (MTCC 227)	65	150	200
2	<i>Aspergillus niger</i> (NCIM 501)	65	150	200

Table 12: Minimum Inhibition Concentration for the methanol and aqueous extract of *M. balsamina* Linn. Fruit

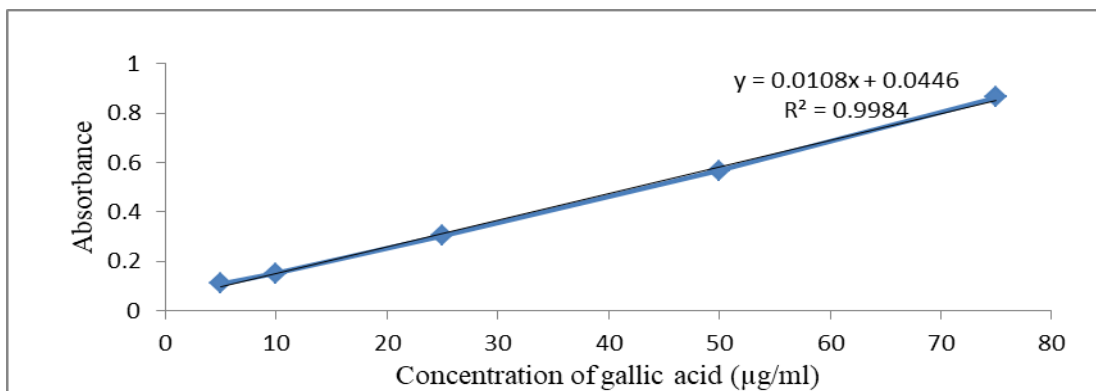


Figure 1: Calibration curve of Gallic acid

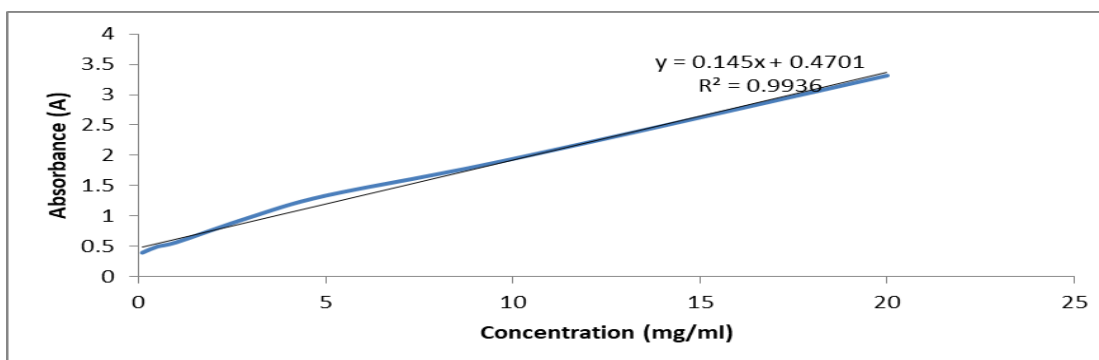


Figure 2: Calibration curve of quercetin

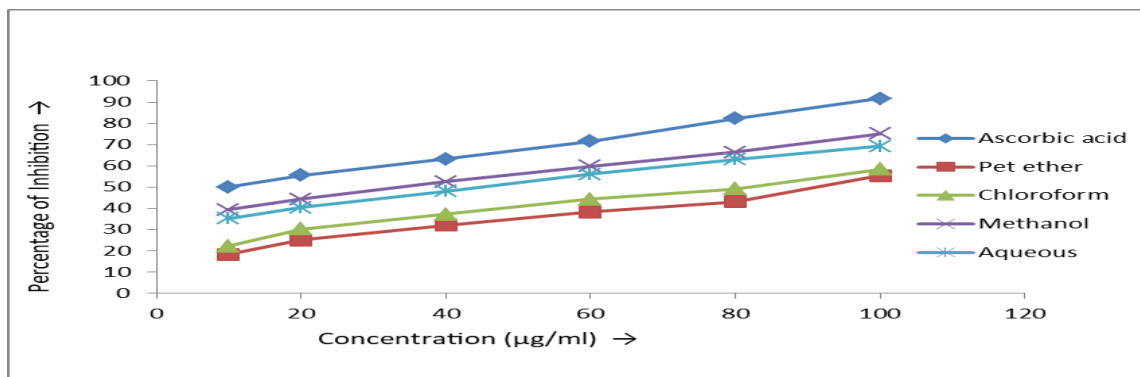


Figure 3: Effect of *M. balsamina* Linn. Fruits extracts on DPPH Radical Scavenging activity

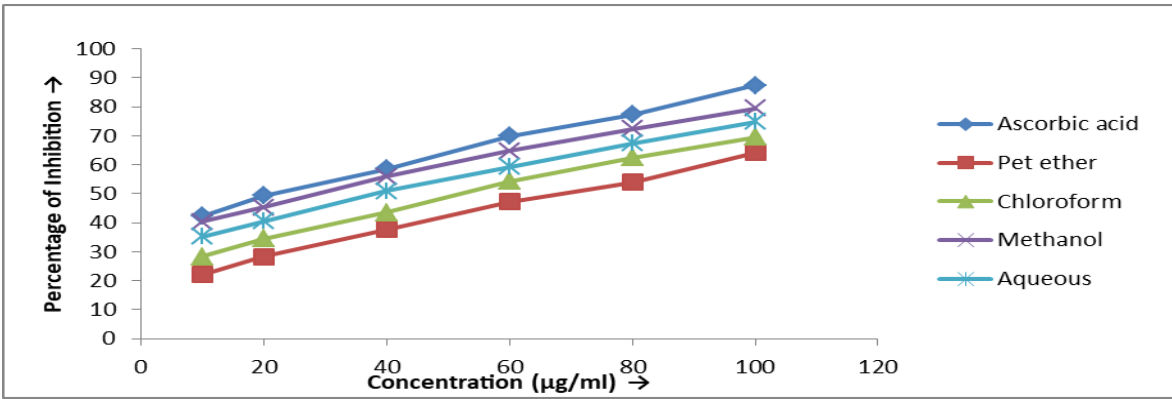


Figure 4: Effect of Momordica balsamina Linn. Fruit extracts on Hydrogen Peroxide Scavenging activity

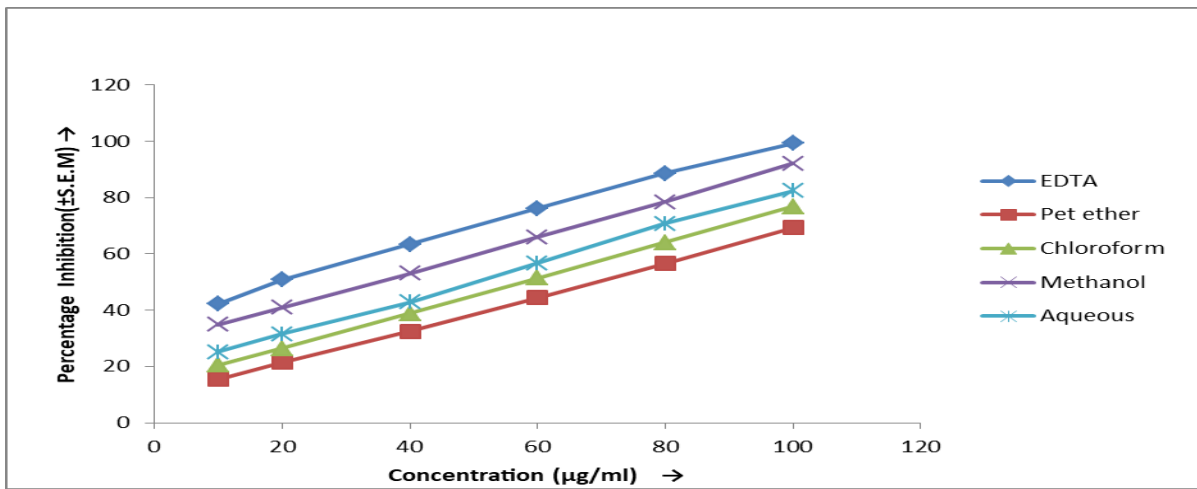


Figure 5: Effect M. balsamina Linn. Fruit extracts on reducing power assay

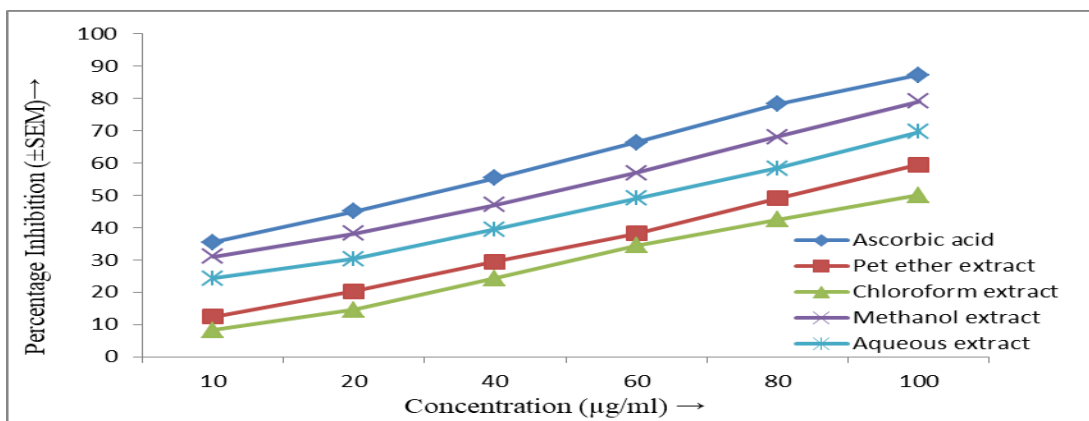


Figure 6: Effect of M. balsamina Linn. Fruit extracts on ferrous chelating ability

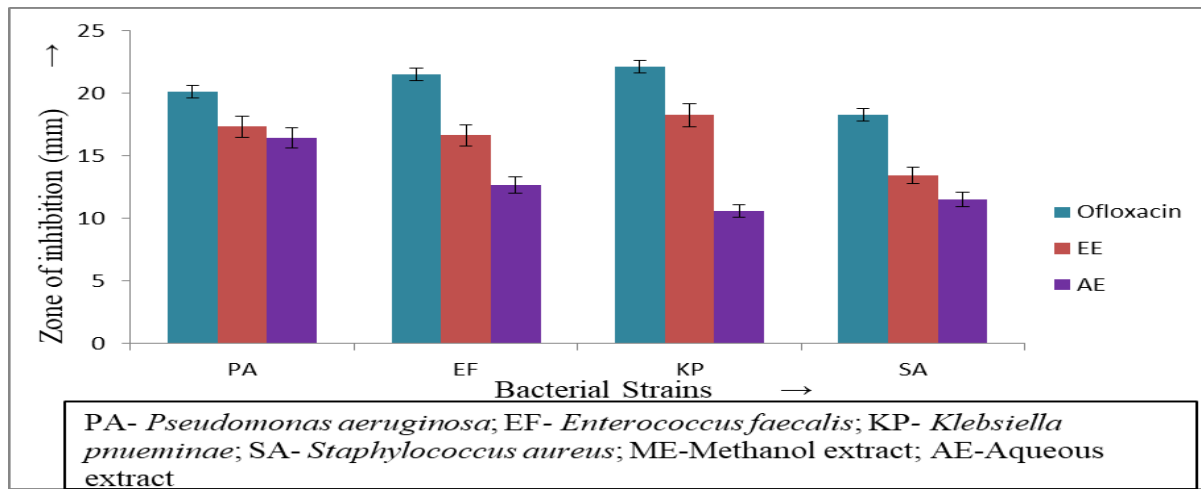


Figure 7: Percentage of the zone of inhibition for the methanol and aqueous extract of *M. balsamina* Linn. Fruit.

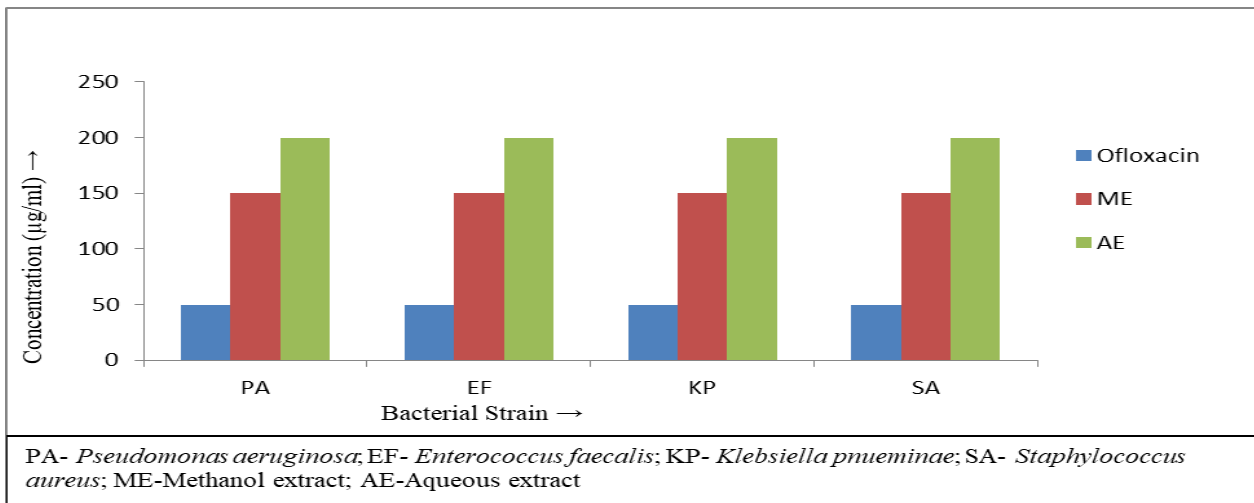


Figure 8: Minimum Inhibition Concentration for the methanol and aqueous extract of *M. balsamina* Linn. Fruit

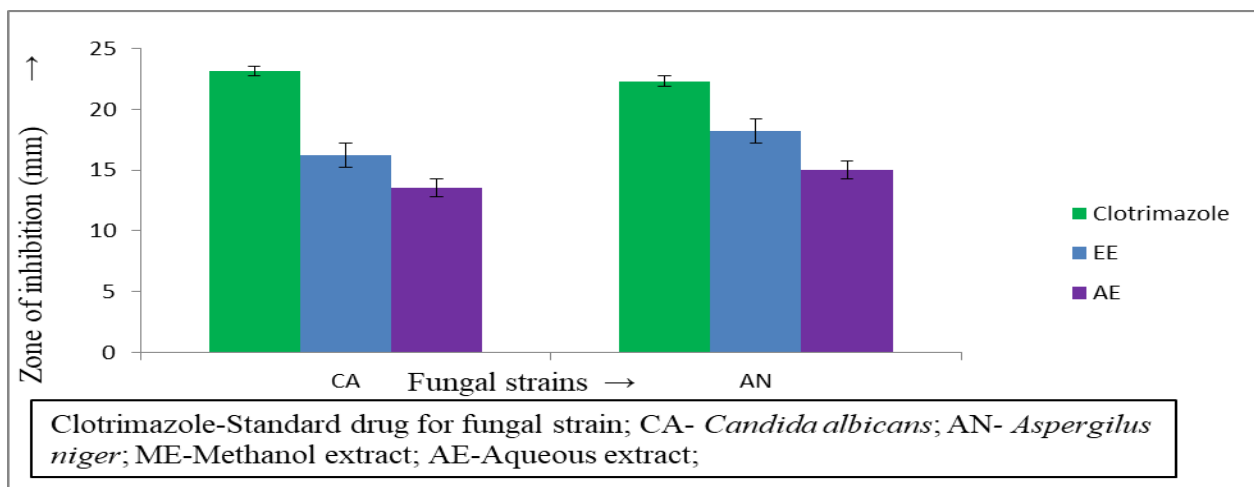


Figure 9: Percentage of the zone of inhibition for the methanol and aqueous extract of *M. balsamina* Linn fruit

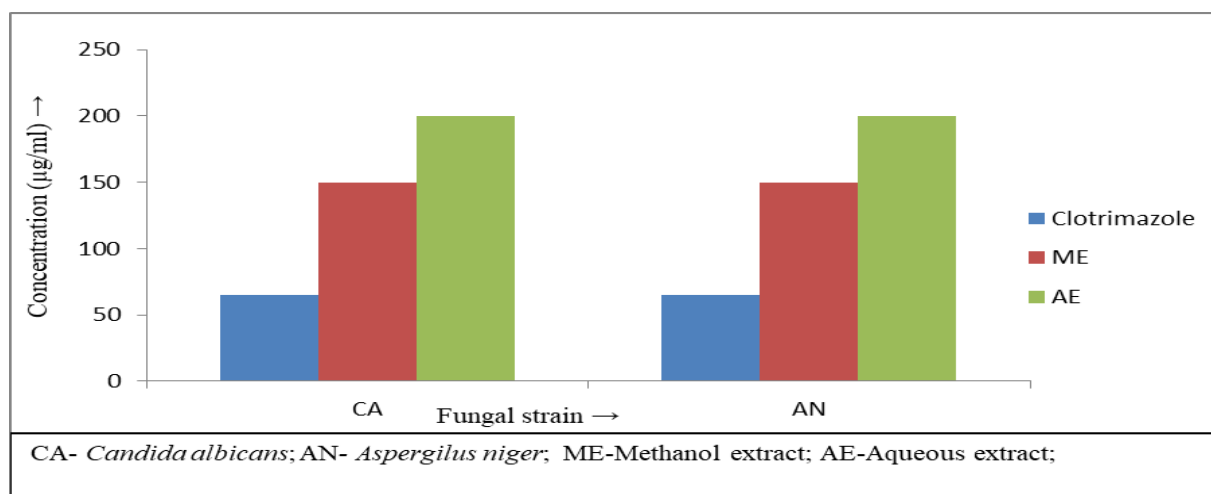


Figure 10: Minimum Inhibition Concentration for the methanol and aqueous extract of *M.balsamina* Linn fruit

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