

**COMPARATIVE PHYTOCHEMICAL EVALUATION,
INVESTIGATION AND ACTIVITY OF MORINGA OLEIFERA
LEAVES AND FLOWERS EXTRACT**

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ABSTRACT

Moringa oleifera plant has nutritional contents which are diseases preventing and it has been used from ancient age in the treatment, prevention of disease or infection. The essential vitamins and minerals are used to prevent and cure the diseases and to save the populations from microbial infection and that may be a most potent antimicrobial medicine from natural source. So it is important to study the phytoconstituents of different part of the plant. The plant have been used for various diseases in the folk medicine and the secondary metabolites is the most important bioactive constituents which include alkaloids, phenolic compounds, tannins, phytosterols and terpenoids are the most popular phytomedicine works instead of antibiotics and that have lesser side effects or adverse effects or other hypersensitive reactions or allergic reactions. So, there have possibility of good

antimicrobial and antifungal activity. Now there have many studies available on leaves extract but there has no sufficient study on flower. So the comparable study of the leaves and flower constituents is beneficial for future. In the sense the plant has a rich polyphenolic compounds so there may have most useful antioxidant activity.

KEYWORDS: *Moringa Oleifera*, Nutritional values, pharmacological benefits, leaves, flowers.

INTRODUCTION

Moringa oleifera Lam. is a plant commonly popular by name as drumstick plant or horse radish plant or miracle plant or mother's best friend and it is the most widely cultivated

species of the genus *Moringa* and the family *Moringaceae*.^[1] In a single genera it has 33 species of which 4 are accepted and over all 13 species are documented and available that are native to old world tropics.^[2] The *M.oleifera* plant is known by more than 80 countries and it has been popular in more than 200 local languages by various societies including the Roman, Greek, Egypt, India and many other country for thousands of years back as 150 AD and ancient Maurian period at the time of 150 BC.^[3] *M.oleifera* is one of the most useful tropical trees that have greater nutritional value as well as multi-therapeutic activities and widely cultivated the leaves, oil and flowers in traditional herbal medicine in South -East Asian and African countries .It is fast growing evergreen tree that usually grows up to 10 to 12 meters in height with fragile branches and trip innate leaves and thick corky, whitish bark.^[3-4]

The *Moringa Oleifera* plants have remarkable nutritional and medicinal values and after analysis it appears to have the micro-nutrient content is even more in dried leaves; (ten times the vitamin A of carrots), (17 times the calcium of milk), (15 times the potassium of bananas), (25 times the iron of spinach) and (nine times the protein of yogurt) (Trees for Life, 3006 W. St. Louis, Wichita, KS 67203-5129 USA., section II scientific data page no. 5).And the tree have been used in the purpose of malnutrition for infants and nursing mothers from ancient period and the leaves can be eaten fresh, cooked or stored as dried powder for many months without refrigeration and without loss of the actual nutritional values. The *Moringa Oleifera* plants have a remarkable high amount of vitamin C that used in illness including colds and flu. Vitamin A used in the treatment of eye disease, skin disease, heart ailments, diarrhoea, and many other diseases. Calcium is essential for strong bones and teeth and helps prevent osteoporosis .Potassium is essential for the functioning of the brain and nerves. Proteins are the basic need for all our body cells.

The role of essential amino acid obtained from *Moringa* leaves are most important need of protein synthesis in the body that is argenine and histidine are especially important for infants, who are unable to make enough protein for their growth requirements. And now a day's there have so many useful plant product from *Moringa* leaves such as Moringa Tea, Moringa Tablets, Moringa Capsules, Moringa leaf Powder, Moringa Soaps and Moringa Face wash. Recently, a few others like *M. stenopetala*, *M. peregrina* and *M. concanensis* have been discovered to be having equal potential such as nutritious vegetables, high-quality seed oil, antibiotics and water clarification agents just like the *M. oleifera*.^[5-6]

The plant extract may interact with cytochrome P450 enzymes. The leaves extract have anti-amnesiac properties. It may inhibit cholesterol absorption from intestines and may be a hypolipidemic effect of supplementation in diabetics. *Moringa oleifera* have potential inhibitory effects on sucrose absorption although no apparent inhibition of starch absorption but appears to have hypoglycaemic properties. It may protective against neutrophil related immunosuppression effects and have anti-inflammatory and anti-edemic properties. The leaves extracts have somewhat responsive antibacterial properties and it may capable of reducing microcystin. It has responsive antioxidant properties and leaves may have a suppressive effect on the conversion of T4 into active T3. The seed extracts have anti-cholinergic and anti-inflammatory properties in the airway disorder that may suggest anti-asthmatic properties. *Moringa oleifera* works on 5-HT3 receptors in stomach that exert protective effects on ulceration and leaf extracts acts on kidney where it may protect against oxidative toxins. The plants have some diuretic properties and it may appear to have anti-cancer properties in vitro against pancreatic cancer in higher concentration. Leaves extracts have anticoagulant properties and that may possess wound healing properties. [5-10]

Table: 1 Taxonomical Classification^[7]

| | | | |
|----------------|----------------|------------------|--------------------|
| Kingdom | Plantae | Sub class | Dilleniidae |
| Sub Kingdom | Tracheobionta | Order | Brassicales |
| Super Division | Spermatophyta | Family | Moringaceae |
| Division | Magnoliophyta | Genus | Moringa |
| Class | Magnoliopsida | Species | Oleifera |

Table: 2 Botanical description and local names^[7]

| Language | Synonyms | Language | Synonyms | Language | Synonyms |
|----------|--|------------|----------------------|-----------|---------------------|
| Latin | Moringa oleifera | Portuguese | Moringa, Moringueiro | Tamil | Morigkai , murungai |
| Sanskrit | Subhanjana , shobhanjana | Bengali | sajina | Telugu | Mulaga, |
| English | Drumstick tree, Horseradish tree, Ben tree | Hindi | Saguna, Sainjna | Malayalam | Murinna, Sigru |

Importance of the plant

Moringa oleifera plant has nutritional contents which are diseases preventing and it has been used from ancient age in the treatment, prevention of disease or infection. The essential vitamins and minerals are used to prevent and cure the diseases and to save the populations from microbial infection and that may be a most potent antimicrobial medicine from natural source. So it is important to study the phytoconstituents of different part of the plant. The plant have been used for various diseases in the folk medicine and the secondary metabolites

is the most important bioactive constituents which include alkaloids, phenolic compounds, tannins, phytosterols and terpenoids are the most popular phytochemistry works instead of antibiotics and that have lesser side effects or adverse effects or other hypersensitive reactions or allergic reactions. So, there have possibility of good antimicrobial and antifungal activity. Now there have many studies available on leaves extract but there has no sufficient study on flower. So the comparable study of the leaves and flower constituents is beneficial for future. In the sense the plant has a rich polyphenolic compounds so there may have most useful antioxidant activity.

MATERIALS AND METHODS

1. In-vitro Antibacterial activity.

Collection and authentication of the plant

The leaves and flowers of *Moringa Oleifera* Lam were collected from Murshidabad district of West Bengal, India in the month of January 2015. The plant were taxonomically identified by BB college Asansol, Burdwan, India(No.- 122 BBC/BOT/2015-16) and the voucher specimen were retained in our laboratory for future reference.

Table-3 Antibacterial activity of ethanolic *Moringa Oleifera* leaves and flowers extract against (gram positive) bacteria.

| Name of Bacteria | Control | Conc. of <i>M. Oleifera</i> leaves extract(mg/ml) | | | | Conc. of <i>M. Oleifera</i> Flowers extract(mg/ml) | | | |
|--------------------------------|---------|---|-----|---|----|--|-----|---|----|
| | | 1.25 | 2.5 | 5 | 10 | 1.25 | 2.5 | 5 | 10 |
| <i>S.aureus</i> ML-276 | + | + | + | - | - | + | + | - | - |
| <i>S.aureus</i> ATCC-25923 | + | + | + | - | - | + | + | + | - |
| <i>S.aureus</i> ML-152 | + | + | + | - | - | + | + | - | - |
| <i>S.aureus</i> ML-281 | + | + | + | - | - | + | + | - | - |
| <i>S.aureus</i> ATCC-29157 | + | + | + | + | - | + | + | + | - |
| <i>S.aureus</i> 8531 | + | + | + | - | - | + | + | - | - |
| <i>B.licheniformis</i> 10341 | + | + | + | - | - | + | + | - | - |
| <i>B.polymyxa</i> 4747 | + | + | + | - | - | + | + | - | - |
| <i>B. subtilis</i> (ATCC 6633) | + | + | + | - | - | + | + | + | - |
| <i>S.aureus</i> ML-351 | + | + | + | - | - | + | + | - | - |

(+)=Growth positive ;(-) =Growth negative.

Leaves and flowers of *Moringa Oleifera* have promising antimicrobial activity which inhibited the most gram positive bacteria used in the experiment within 5mg/ml to 10mg/ml concentration of leaves and flowers extract of *Moringa Oleifera*. Minimum Inhibitory

Concentration (MIC) was indicated by the lowest concentration of the extract which inhibited respective bacterial growth.

Table: 4 Antibacterial activity of ethanolic *Moringa Oleifera* leaves and flowers extract against (gram negative) bacteria.

| Name of Bacteria | Control | Conc. of <i>M. Oleifera</i> leaves extract(mg/ml) | | | | Conc. of <i>M. Oleifera</i> Flowers extract(mg/ml) | | | |
|----------------------------|---------|---|-----|---|----|--|-----|---|----|
| | | 1.25 | 2.5 | 5 | 10 | 1.25 | 2.5 | 5 | 10 |
| <i>Shigella sonar DN3</i> | + | + | + | + | - | + | + | + | - |
| <i>V.cholerae 793</i> | + | + | + | - | - | + | + | - | - |
| <i>S.typhimuriumNCTC74</i> | + | + | + | + | - | + | + | + | - |
| <i>S.typhi TY259</i> | + | + | + | - | - | + | + | + | ± |
| <i>S.flexneri 2DDN3</i> | + | + | + | - | - | + | + | + | - |
| <i>S.typhi D7372</i> | + | + | + | ± | - | + | + | ± | - |
| <i>E.coli ATCC 25922</i> | + | + | + | + | + | + | + | + | + |
| <i>S.typhi 59</i> | + | + | + | + | - | + | + | + | - |
| <i>V.cholerae 71</i> | + | + | + | + | + | + | + | + | + |
| <i>S.flexneri 4924</i> | + | + | + | + | - | + | + | + | - |

(+)=Growth positive ; (-) =Growth negative.

Leaves and flowers of *Moringa Oleifera* have promising antimicrobial activity which inhibited the most gram negative bacteria used in the experiment within 5mg/ml to 10mg/ml concentration of leaves and flowers extract of *Moringa Oleifera*. Minimum Inhibitory Concentration (MIC) was indicated by the lowest concentration of the extract which inhibited respective bacterial growth.

Preparation of Inoculums

The gram positive (*Bacillus subtilis* and *Staphylococcus aureus*) and gram negative bacteria (*Escherichia coli*, *Salmonella typhimurium*, *Salmonella typhi*, *Vibrio cholera*) Were pre-cultured in nutrient broth and peptone broth overnight in Bijou bottles and kept it in BOD incubator at 37°C.

Preparation of the Standard Solution

To prepare a stock solution, dissolve a quantity of the Standard Preparation of a given antibiotic, accurately weighed and previously dried, and then dilute to the required concentration (50 mg in 100ml i.e.500 µg/ml) as indicated. Store in a refrigerator and use within the period indicated.

Preparation of the solutions of extract (i.e. sample solution):The concentration of the extracts used for this experiment was 10 mg/ml. The ethanolic extract was prepared in water medium.

Table: 5 Zone of Inhibition to determine antibacterial activity of the extract and standard antibiotics

| Bacterial sp. | Zone of inhibition (mm) <i>Moringa Oleifera</i> | | | |
|-------------------------------------|---|----------------------|----|-----------------|
| | Leaves extract | Standard Antibiotics | | Flowers extract |
| | | Cp | Am | |
| <i>S.aureus ML-281</i> | 13 | 23 | 21 | 10 |
| <i>S.aureus ML-276</i> | 10 | 27 | 18 | 8 |
| <i>S.aureus ML-152</i> | 14 | 23 | 22 | 11 |
| <i>Bacillus subtilis (ATCC6633)</i> | 12 | 27 | 24 | 10 |
| <i>S.typhimurium NCTC 74</i> | 14 | 28 | 20 | 11 |
| <i>E.coli ATCC 25922</i> | 11 | 24 | 18 | 9 |
| <i>S.typhi 59</i> | 12 | 28 | 18 | 8 |
| <i>V.cholerae 71</i> | 9 | 23 | 19 | 8 |

Values are mean inhibition zone (mm) and average of three replicates.

Here, Cp = Ciprofloxacin.

Am= Amoxicillin

The conc. of above drugs is 5 µg /cylinder)

Moringa Oleifera (1mg/cylinder)

DISCUSSION

From the table, it can be concluded that the leaves and flowers extract of *Moringa Oleifera* have promising antibacterial activity.

Combination Antibacterial effect of *Moringa Oleifera* with Ciprofloxacin

Table: 6 Combination Antibacterial effect of *Moringa Oleifera* with Ciprofloxacin

| Name of Bacteria | Zone of inhibition (mm) <i>M. Oleifera</i> Leaves and flowers | | | | |
|-------------------------------|---|-----|----|-----------------|---------|
| | Individual effect | | | Combined effect | |
| | M.L | M.F | CP | M.L+ CP | M.F+ CP |
| <i>S.aureus ML-152</i> | 15 | 14 | 23 | 27 | 14 |
| <i>B. subtilis (ATCC6633)</i> | 13 | 12 | 27 | 29 | 13 |
| <i>S.typhimurium NCTC 74</i> | 16 | 14 | 28 | 30 | 15 |
| <i>S.typhi 59</i> | 13 | 12 | 28 | 28 | 13 |

Values are mean inhibition zone (mm) and average of three replicates.

M.L means *Moringa Oleifera* leaves

M.F means *Moringa Oleifera* flowers

CP means Ciprofloxacin

Moringa Oleifera and Ciprofloxacin are synergistic to *S.aureous* ML-152, *Bacillus subtilis* (PZ6633), *S.typhimurium* NCTC 74, *S.typhi* 59 which can be recognized as a potent antibacterial combination.

Combination Antibacterial effect of *Moringa Oleifera* with Amoxicillin

Table: 7 Combination Antibacterial effect of *Moringa Oleifera* with Amoxicillin

| Name of Bacteria | Zone of inhibition (mm) <i>M. Oleifera</i> Leaves and flowers | | | | |
|-------------------------------|---|-----|----|-----------------|---------|
| | Individual effect | | | Combined effect | |
| | M.L | M.F | Am | M.L+ Am | M.F+ Am |
| <i>S.aureus</i> ML-152 | 14 | 13 | 22 | 24 | 15 |
| <i>B. subtilis</i> (ATCC6633) | 12 | 11 | 21 | 23 | 14 |
| <i>S.typhimurium</i> NCTC 74 | 14 | 13 | 23 | 25 | 15 |
| <i>S.typhi</i> 59 | 12 | 10 | 18 | 22 | 13 |

Values are mean inhibition zone (mm) and average of three replicates.

M.L means *Moringa Oleifera* leaves

M.F means *Moringa Oleifera* flowers

Am means Amoxicillin

Moringa Oleifera and Amoxicillin are synergistic to *S.aureous* ML-152, *Bacillus subtilis* (PZ6633), *S.typhimurium* NCTC 74, *S.typhi* 59 which can be recognized as a potent antibacterial combination.

Fractional Inhibitory Concentration (FIC) Index Determination

(Checker board technique)

Ethanollic leaves extract of *Moringa Oleifera* with Ciprofloxacin^[11]

The medium used in this experiment was Mueller Hinton broth, the drugs tested were ethanolic leaves extract of *Moringa Oleifera* and Ciprofloxacin and the test organism was *S.typhimurium* NCTC 74. The MIC of ethanolic leave extract of *Moringa Oleifera* with respect to this organism was 5 mg/ml and that of ciprofloxacin was 5 µg/ml. The concentrations tested of these two drugs as follows.

Isobologram:

The medium used in this experiment was Mueller Hinton broth; the drugs tested were ethanolic leaves extract of *Moringa Oleifera* and Ciprofloxacin and the test organism was *S.typhimurium* NCTC 74.

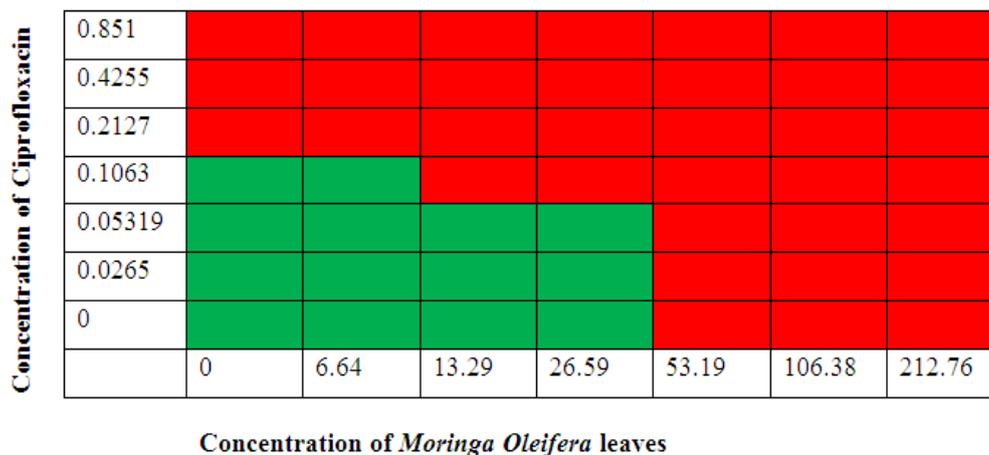


Figure: 1 Isobologram

(Green colour)=Growth positive ;(red colour) =Growth negative.MIC of ethanolic leaves extract of *M.Oleifera* alone to the organism was 10mg/ml and the MIC of the ciprofloxacin alone to the organism was 5 µg.

RESULTS

From the table, the fractional inhibitory concentrations (FIC) index was calculated according to the following formula.

Here, (A) = 0.265 µg/ml

(MIC_A) = 0.1063 µg/ml

(B) = 53.19 µg/ml

(MIC_B) = 212.76 µg/ml

So all the data here we given to the following formula.

$$(A)/ (MIC_A) + (B)/ (MIC_B) = FIC_A + FIC_B$$

$$= FIC_{index}$$

$$\text{Or, } (0.265/ (0.1063) + (53.19/212.76) = FIC_A + FIC_B$$

$$\text{Or, } FIC_A + FIC_B = 0.2492 + 0.25$$

$$\text{Or, } FIC_{index} = 0.492 (< 0.50)$$

The fractional inhibitory concentration (FIC) index was calculated according to the above formula was 0.492.

2. In -Vitro Antifungal Activity

Table-8 Name of Fungi

| Name of Fungi | Strain |
|------------------------------|------------|
| <i>Candida tropicalis</i> | ATCC 750 |
| <i>Candida albicans</i> | ATCC 10231 |
| <i>A.niger</i> | ATCC 1056 |
| <i>P.notatum</i> | ATCC 11625 |
| <i>Candida parapsiliasis</i> | ATCC 1032 |

Standard Drug

Table: 9 Name of standard drug

| Name of the Drug | Abbreviation | Sources |
|------------------|--------------|---------------------------------------|
| Fluconazole | Fz | Abbott Laboratories (India) Pvt. Ltd. |

Preparation of test sample

50 mg extract is suspended in 5ml double distilled water which concentration is 10mg/ml (A).It is kept as stock solution. From which further subsequent dilution has been done as 5mg/ml (B), and 2.5mg/ml (C), 1.25mg/ml (D).

Table: 10 Antifungal activity of ethanolic *Moringa Oleifera* leaves and flowers extract against fungi.

| Name of Fungi | Control | Conc. of <i>Moringa Oleifera</i> leaves extract(mg/ml) | | | | Conc. of <i>Moringa Oleifera</i> Flowers extract(mg/ml) | | | |
|------------------------------|---------|--|-----|---|----|---|-----|---|----|
| | | 1.25 | 2.5 | 5 | 10 | 1.25 | 2.5 | 5 | 10 |
| <i>Candida tropicalis</i> | + | + | + | - | - | + | + | - | - |
| <i>Candida albicans</i> | + | + | + | ± | ± | + | + | ± | ± |
| <i>A.niger</i> | + | + | + | - | - | + | + | + | - |
| <i>P.notatum</i> | + | + | + | - | - | + | + | - | - |
| <i>Candida parapsiliasis</i> | + | + | + | ± | - | + | + | ± | - |

(+)=Growth positive ;(-) =Growth negative.

Leaves and flowers of *Moringa Oleifera* have promising antifungal activity which inhibited the different fungi used in the experiment within 5mg/ml to 10mg/ml concentration of leaves and flowers extract of *Moringa Oleifera*. Minimum Inhibitory Concentration (MIC) was indicated by the lowest concentration of the extract which inhibited respective fungal growth.

Disc diffusion technique**Table: 11 Zone of inhibition to determine antifungal activity of the extract and standard antibiotics.**

| Fungi sp. | Zone of inhibition (mm) of <i>Moringa Oleifera</i> | | |
|------------------------------|--|---------|----------------------|
| | Leaves | flowers | Std. Antibiotics(Fz) |
| <i>Candida tropicalis</i> | 12 | 11 | 14 |
| <i>Candida albicans</i> | 15 | 12 | 17 |
| <i>A.niger</i> | 13 | 10 | 18 |
| <i>P.notatum</i> | 11 | 9 | 17 |
| <i>Candida parapsiliasis</i> | 10 | 8 | 15 |

Values are mean inhibition zone (mm) and average of three replicates.

Here, Fz = Fluconazole.

The conc. of above drugs is 5 µg /cylinder)

Moringa Oleifera (1mg/cylinder)

DISCUSSION

From the table, it can be concluded that the leaves and flowers extract of *Moringa Oleifera* have promising antifungal activity against *Candida tropicalis*, *Candida albicans*, *A.niger*.

Combination Antifungal effect of *Moringa Oleifera* with standard antifungal drug Fluconazole.**Table: 12 Zone of inhibition for combination antifungal effect.**

| Name of Fungi | Zone of inhibition (mm) <i>M. Oleifera</i> Leaves and flowers extract | | | | |
|---------------------------|---|-----|----|-----------------|---------|
| | Individual effect | | | Combined effect | |
| | M.L | M.F | Fz | M.L+ Fz | M.F+ Fz |
| <i>Candida tropicalis</i> | 12 | 10 | 15 | 15 | 14 |
| <i>Candida albicans</i> | 10 | 9 | 17 | 17 | 16 |
| <i>A.niger</i> | 12 | 10 | 15 | 14 | 13 |

Values are mean inhibition zone (mm) and average of three replicates.

M.L means *Moringa Oleifera* leaves

M.F means *Moringa Oleifera* flowers

Fz means Fluconazole

From the above table it can be concluded that *Moringa Oleifera* leaves and flowers extract and Fluconazole are not synergistic and which could not be recognized as a potent antifungal combination.

RESULTS

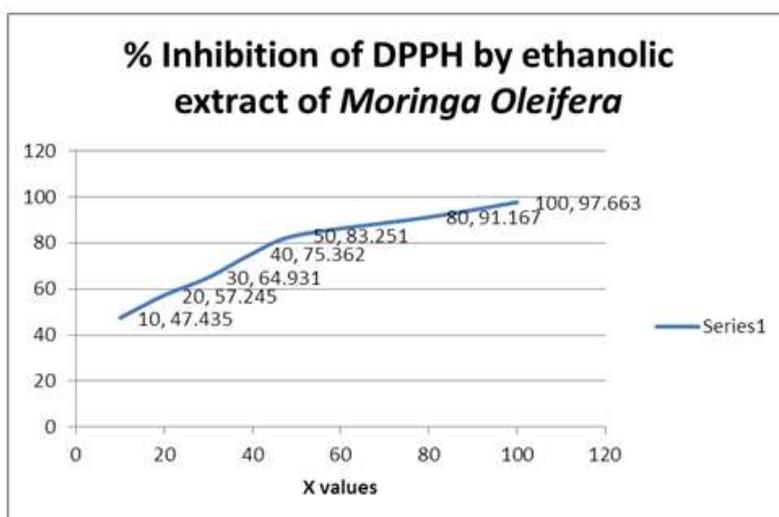
Table-13 DPPH radical scavenging activity of ethanolic flower extract of *Moringa Oleifera*.

| Conc. of ethanol extract($\mu\text{g/ml}$) | Absorbance | | | | % Inhibition | | | |
|--|------------|-------|-------|-------|--------------|-------|-------|------------------------|
| | A1 | A2 | A3 | DPPH | I | II | III | Average \pm SD |
| 10 | 0.639 | 0.641 | 0.639 | 0.701 | 8.845 | 8.559 | 8.845 | 8.75 \pm 0.165 |
| 20 | 0.586 | 0.591 | 0.586 | | 16.41 | 15.69 | 16.41 | 16.17 \pm 0.412 |
| 30 | 0.512 | 0.501 | 0.512 | | 26.96 | 28.53 | 26.96 | 27.48 \pm 0.91 |
| 40 | 0.472 | 0.481 | 0.472 | | 32.67 | 31.38 | 32.67 | 32.24 \pm 0.741 |
| 50 | 0.419 | 0.409 | 0.419 | | 40.23 | 41.65 | 40.23 | 40.71 \pm 0.823 |
| 80 | 0.358 | 0.362 | 0.358 | | 48.93 | 48.36 | 48.93 | 48.74 \pm 0.329 |
| 100 | 0.287 | 0.293 | 0.287 | | 59.06 | 58.20 | 59.06 | 58.77 \pm 0.494 |
| 200 | 0.093 | 0.099 | 0.093 | | 86.73 | 85.88 | 86.73 | 86.45 \pm 0.494 |
| IC ₅₀ | | | | | | | | 89.84 $\mu\text{g/ml}$ |

Table-14: The comparative study of

DPPH radical scavenging activity of ethanolic *Moringa Oleifera* flower extract of *Moringa Oleifera* and standard Ascorbic acid.

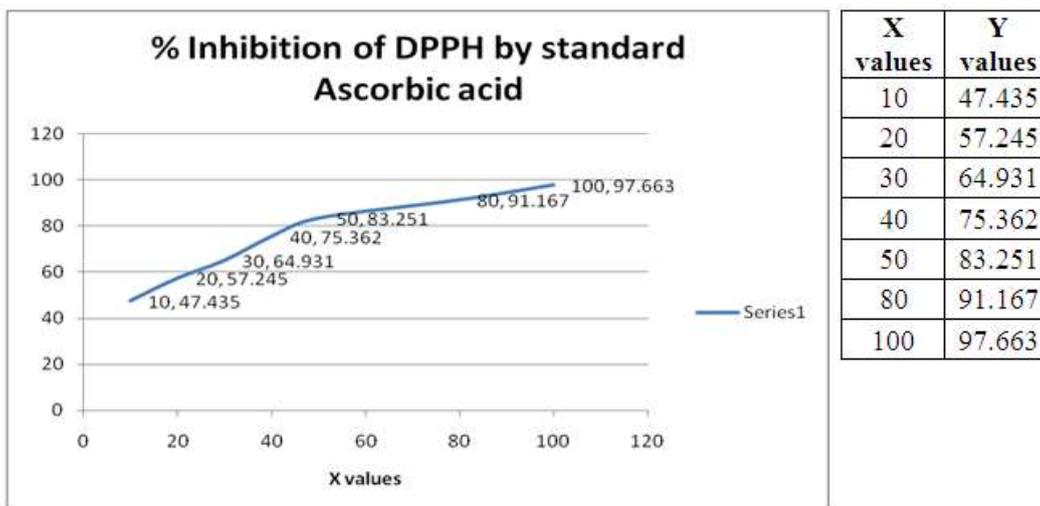
| Serial No. | Concentration ($\mu\text{g/ml}$) | %DPPH Inhibition by ethanolic flower extract of <i>Moringa Oleifera</i> | %DPPH Inhibition by Ascorbic |
|------------|------------------------------------|---|------------------------------|
| | | Mean \pm SD | Mean \pm SD |
| 1 | 10 | 8.75 \pm 0.165 | 47.435 \pm 5.875 |
| 2 | 20 | 16.17 \pm 0.412 | 57.245 \pm 1.255 |
| 3 | 30 | 27.48 \pm 0.91 | 64.931 \pm 0.883 |
| 4 | 40 | 32.24 \pm 0.741 | 75.362 \pm 0.386 |
| 5 | 50 | 40.71 \pm 0.823 | 83.251 \pm 0.274 |
| 6 | 80 | 48.74 \pm 0.329 | 91.167 \pm 0.263 |
| 7 | 100 | 58.77 \pm 0.494 | 97.663 \pm 0.195 |



| X values | Y values |
|----------|----------|
| 10 | 8.75 |
| 20 | 16.17 |
| 30 | 27.48 |
| 40 | 32.24 |
| 50 | 40.71 |
| 80 | 48.74 |
| 100 | 58.77 |
| 200 | 86.45 |

IC₅₀ value=89.84 mcg/ml

Figure: 2 Percentage Inhibition of DPPH by ethanolic extract of *M. Oleifera*



IC₅₀ value=12.045 mcg/ml

Figure: 3 Percentage Inhibition of DPPH by standard Ascorbic acid

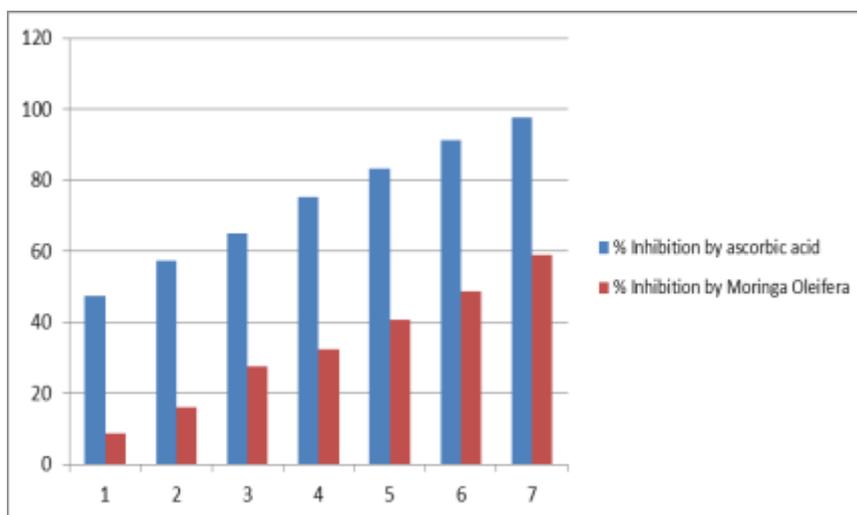


Figure: 4 Comparative of % DPPH inhibition capacity between Ascorbic acid and ethanolic flower extract of *Moringa Oleifera*.

Nitric oxide radical scavenging assay:

Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide which interacts with oxygen to produce nitrite ions, which can be measured at 546 nm spectrophotometrically in the presence of Griess reagent.

Reagents used

Sodium nitroprusside [40190,S.d.fine Chemicals, India], Sulphanilamide[3164,NR Chem., India], Orthophosphoric acid [39416,S.d.fine Chemicals, India],N-(1-naphthyl) ethylene

diamine [N5889,S.d.fine Chemicals, India],Positive control: Curcuminoids (33533,Synthite,Kochin,India).

Procedure

Test solution of various concentrations (1ml) and 1ml of 10 M sodium nitroprusside are illuminated (using fluorescence light/18W CDL 6500K) at room temperature (25-30⁰C) for 15 min. Following incubation, 1ml of Griess reagent was added and incubated for 10 min. At room temperature the colour developed was measured at 546 nm. Linear graph of concentration Vs percentage inhibition was prepared and IC₅₀ values were calculated.

RESULTS

Table: 15 Nitric oxide radical scavenging activity of ethanolic flower extract of *Moringa Oleifera*.

| Conc. of ethanol extract(µg/ml) | Absorbance | | | | % Inhibition | | | |
|---------------------------------|------------|-------|-------|-------|--------------|---------|-------------|-------------|
| | A1 | A2 | A3 | DPPH | I | II | III | Average ±SD |
| 10 | 0.372 | 0.371 | 0.370 | 0.397 | 6.29723 | 6.54912 | 6.80101 | 6.55±0.25 |
| 20 | 0.329 | 0.328 | 0.325 | | 17.1285 | 17.3804 | 18.136 | 17.54±0.52 |
| 30 | 0.286 | 0.285 | 0.290 | | 27.9597 | 28.2116 | 26.9521 | 27.71±0.67 |
| 40 | 0.228 | 0.225 | 0.229 | | 42.5693 | 43.3249 | 42.3174 | 42.74±0.53 |
| 50 | 0.209 | 0.211 | 0.205 | | 47.3552 | 46.8514 | 48.3627 | 47.52±0.77 |
| 80 | 0.179 | 0.175 | 0.180 | | 54.9118 | 55.9194 | 54.6599 | 55.16±0.67 |
| 100 | 0.160 | 0.158 | 0.161 | | 59.6977 | 60.2015 | 59.4458 | 59.78±0.38 |
| 200 | 0.136 | 0.137 | 0.139 | | 65.7431 | 65.4912 | 64.9874 | 65.41±0.63 |
| IC ₅₀ | | | | | | | 68.75 µg/ml | |

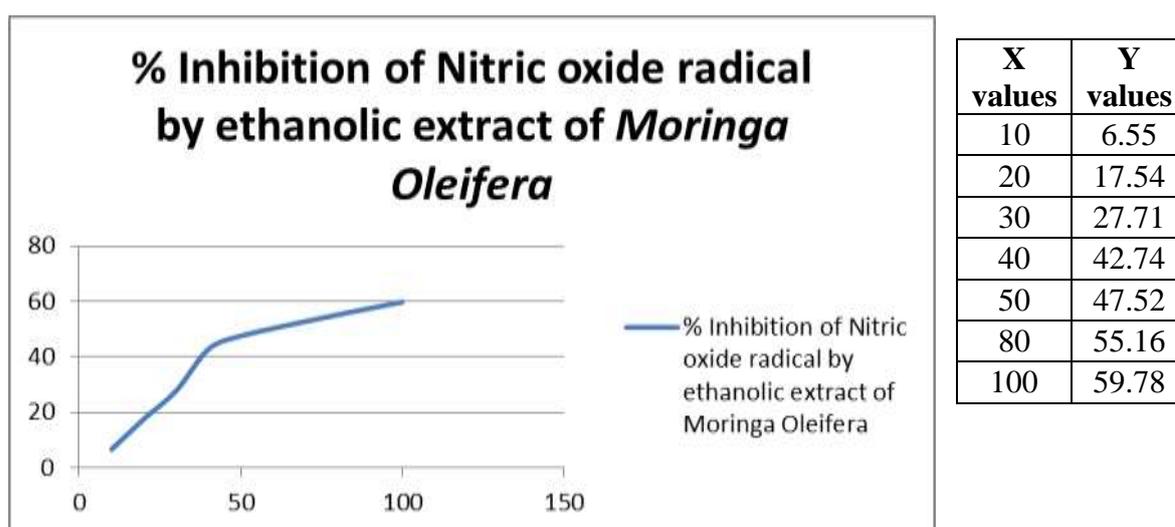


Figure: 5 Percentage Inhibition of Nitric oxide radical by ethanolic extract of *Moringa Oleifera*.

Y axis stands for percentage inhibition and X axis stands for concentration.

CONCLUSION

From the above result it can be concluded that the fractional inhibitory concentrations (FIC) index was 0.492 (< 0.50) that means *Moringa Oleifera* and Ciprofloxacin are synergistic to *S.typhimurium* NCTC 74. And also it can be concluded that *Moringa Oleifera* has promising antibacterial activity.

DISCUSSION

As illustrated above figures the ethanolic flower extract of *Moringa Oleifera* exhibit a concentration-depended DPPH and Nitric oxide radical scavenging capacity. The ethanolic flower extracts of *Moringa Oleifera* exhibited strong DPPH radical scavenging activity but slight lower than positive control. Radical scavenging activities are important due to the deleterious role of free radicals in food and in biological systems. The method is based on the reduction of alcoholic DPPH solution into non-radical from DPPH in the presence of a hydrogen donating antioxidant. The plants have been reported to contain Flavonoids. Many flavonoids compounds have been poses potent antioxidant activity, which vary according to the number and position of hydroxyl groups.

CONCLUSION

In the present study, a preliminary antimicrobial screening was performed by agar dilution technique; where it was found that ethanolic leaves and flowers extract of *Moringa Oleifera* have promising antimicrobial activity which inhibited the most gram positive and gram negative bacteria and fungi used in the project work.

In the next part of this work, where the combination antimicrobial effect of *Moringa Oleifera* and some antibiotics(Ciprofloxacin and Amoxicillin) are synergistic to *S.aureus* ML-152,*Bacillus subtilis* (PZ6633),*S.typhimurium* NCTC 74, *S.typhi* 59 which can be recognized as a potent antibacterial combination. Whereas, *Candida tropicalis*, *A. niger*, *Candida albicans* which could not be recognized as a potent antimicrobial combination.

After that, the fractional inhibitory concentration (FIC) index was 0.492 (<0.50) that means *Moringa Oleifera* leaves and Ciprofloxacin are synergistic to *S.typhimurium* NCTC 74. The ethanolic flowers extract of *Moringa Oleifera* exhibit a concentration-depended DPPH and Nitric oxide radical scavenging capacity. The ethanolic flower extracts of *Moringa Oleifera*

exhibited strong DPPH radical scavenging activity but slight lower than positive control that means it has good antioxidant property.

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