

Nutritive Composition and Antimicrobial Activity of *Moringa stenopetala* (Baker f.) Cufod

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ABSTRACT

Aims: The present study was carried out to determine nutritive composition and to screen antimicrobial activity of leaf extract of *Moringa stenopetala* (Baker f.) Cufod.

Place and Duration of the Study: The study was performed at Wollega University, Ethiopia, BGK Life Care Technologies Pvt. Ltd., Bagalkot and Department of Microbiology, S.R.N.M.N College of Applied Sciences, Karnataka, India between May-2016 to July-2016.

Methodology: The powdered leaf material was subjected to determination of various proximate parameters namely moisture, ash, crude fibre, crude fat, protein and carbohydrate content. Mineral analysis was carried out using ICP-OES technique. For determining antimicrobial activity, extraction of shade dried and powdered leaf material was carried out by maceration process. Antibacterial activity of extract was assessed against a panel of Gram positive and Gram negative bacteria by Agar well diffusion assay. Antifungal activity was tested against three phytopathogenic fungi by Poisoned food technique.

Results: Leaf material of *M. stenopetala* was found to contain appreciable quantity of carbohydrates (42.20%), crude fibre (07.34%) and protein (26.33%) while the crude fat (05.25%) content was lesser. Among minor elements, the iron content was found to be 962.50 ppm and nickel content was found to be 2.89ppm. In case of major elements, potassium (18426.75ppm) content was found to be highest while the content of sodium (1519.68) was less when compared with other major elements. Extract caused dose dependent inhibition of test bacteria with marked activity against Gram positive bacteria. Extract was not effective against *Ralstonia solanacearum* and showed no activity when tested up to 50mg/ml. A dose dependent suppression of mycelial growth of test fungi was observed in plates poisoned with different concentration of extract.

Conclusion: The leaf of *M. stenopetala* is a good source of proteins, carbohydrates and minerals needed for normal physiology of the body and hence, the leaves can be used as food or feed supplement to enhance growth and health. The plant can be used against infectious microorganisms as the extract exhibited marked antibacterial and antifungal activity.

Keywords: *Moringa stenopetala*; proximate; minerals; maceration; agar well diffusion; poisoned food technique

1. INTRODUCTION

The genus *Moringa* belonging to the family Moringaceae consists of tropical plant species and consists of about 10 species. Of the species of *Moringa*, only *M. oleifera* has got research and development attention. *Moringa* is a multipurpose tree with significant economic value due to its industrial, nutritional and medicinal applications. *Moringa stenopetala* (Baker f.) Cufod (called cabbage tree) is an underutilized, fast growing, domesticated *Moringa* species in East African lowlands and indigenous to southern Ethiopia and is an important indigenous vegetable food crop in south western Ethiopia. It is popularly called Shifara or Shalchada in Ethiopia. Tribes such as Gofa, Konso, Burji and Gamo consume the leaves as vegetable especially during the dry season. The plant is well adapted to semi-arid areas. The seeds contain edible oil that can be used for cooking and as salad dressings. The leaves and fruits are consumed as vegetables as they are rich in proteins, essential amino acids, calcium, iron, phosphorous as well as vitamin A and C. The leaves and pods are often used as animal fodder. The high protein content appears to be the promising use of the plant. The plant is grown as boundary or barrier as it serves as a live fence. The species is also grown as intercropping and grown in mixed multi-storey stands with other food crops. The seeds have flocculating property and hence promising as purifying agent in turbid water. Also, the plant appears to be more resistant to insect pests and tolerates draught. The wood is soft and is not usually used for fuel purpose [1-4].

The potential roles of *M. stenopetala* have been investigated by several researchers. Various parts of the plant have been used traditionally as food and to treat malaria, hypertension, asthma, diabetes, common cold, wounds, retained placenta and stomach problem. The extract of leaf and root caused concentration dependent antileishmanial activity in terms of causing abnormal morphologies in promastigotes of *Leishmania donovani* [5]. The ethanol extract of fresh root wood and the acetone extract of dried leaves were found to be active against trypomastigotes of *Trypanosoma brucei* indicating antitrypanosomal activity [6]. The study of Melesse et al. [2] showed the potential effect of leaf meal of *M. stenopetala* on the nutrient intake and growth performance of Rhode island red chicks under tropical climate. The leaf meal was found to be a cheaper alternate for protein supplement. Extracts and fractions obtained from various parts of the plant have shown to exhibit antimicrobial activity [7, 8]. The crude aqueous extract from leaves was found to cause significant fall in blood pressure of experimental animals indicating the potential blood pressure lowering effect [9]. The seed powder was found to be effective in the removal of chromium from tannery wastewater [10]. The study of Sajidu et al. [11] showed the potential of seed powder of *M. stenopetala* to absorb metals namely cadmium, zinc, copper and chromium. It is found that the leaf powder and extracts of *M. stenopetala* exhibit protective effect in terms of anticoccidial activity against *Eimeria tenella* infection in broiler chickens [12]. The hydroalcoholic extract of leaves of *M. stenopetala* was shown to inhibit intestinal α -glucosidase, pancreatic cholesterol esterase and pancreatic lipase activities indicating the possible role in reducing hyperglycemia and hyperlipidemia [13]. It has been shown that crude extract, solvent fractions and chromatographic fractions from leaves of *M. stenopetala* exhibit antihyperglycemic effect in mice [14]. The present study investigates the nutritive composition and antimicrobial activity of leaf and *M. stenopetala*.

2. MATERIAL AND METHODS

2.1 Collection of Plant Material

The fresh *M. stenopetala* leaves were collected from Ambo, Ethiopia. Ambo city is located 112 km south of Addis Ababa, the capital city of Ethiopia. It lies between latitude 8°59'N, longitude 37°51'E and an elevation of 2101 m above sea level.

74 2.2 Proximate Composition of Leaf

75 The powdered leaf was subjected to analyze various proximate parameters namely
76 moisture, total ash, crude fibre, crude fat, protein and carbohydrates. The assay procedures
77 employed by Indrayan et al. [15] and Kambar et al. [16] were followed for the proximate
78 analysis of *M. stenopetala*.

80 2.3 Moisture Content

81 Weight difference method was employed to estimate the content of moisture in the leaf
82 sample. In brief, a known quantity of powdered material taken in a pre-weighed dish (W1)
83 was kept overnight in hot air oven maintained at 100°C. The dish was cooled and weighed
84 (W2). The moisture content was calculated using the formula:

85 Moisture content = $([W2-W1] / \text{weight of material}) \times 100$.

87 2.4 Total Ash

88 10g of leaf material was placed in a pre-weighed silica crucible (W1) and the crucible was
89 heated over a low flame (till complete charring) followed by heating at 600°C in a muffle
90 furnace for about 3–5 hours. The crucible was cooled in desiccator and weighed again (W2).
91 Heating of the crucible was repeated for half an hour and this was repeated consequently till
92 the weight of ash become constant. Total ash content was calculated using the formula:

93
94 Total ash content = $(\text{weight of ash } [W2-W1] / \text{weight of material}) \times 100$.

96 2.5 Crude Fiber

97 2g of moisture and fat-free leaf material was added to 200ml of 1.25% sulfuric acid and
98 boiled for 30 minutes. The content was filtered and the residue was washed with boiling
99 water till no acidic. The residue was treated with 200ml of 1.25% sodium hydroxide and
100 boiled for 30 minutes. The content was filtered, washed with boiling water followed by 1%
101 nitric acid and hot water. The residue thus obtained was taken in a pre-weighed silica
102 crucible (W1), heated at 600°C until complete ashing and the crucible was again weighed
103 (W2). Crude fibre content was calculated using the formula:

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105 Crude fibre content = $(\text{weight of residue } [W2 - W1] / \text{weight of material}) \times 100$.

107 2.6 Crude Fat

108 2g of moisture free leaf powder was subjected to extraction using petroleum ether (boiling
109 point 40-60°C) in a Soxhlet extractor for 24 hours (or until a drop taken from the drippings
110 left no greasy stain on the filter paper). After completion of extraction, solvent was
111 transferred into a pre-weighed (W1) container. The container was kept in hot air oven
112 (100°C) to evaporate solvent. The container was cooled in desiccator and weighed (W2).
113 The difference in the weight was taken as fat content of leaf material. The crude fat (%) was
114 calculated using the formula:

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116 Crude fat (%) = $(\text{Weight of fat } [W2-W1] / \text{Weight of sample taken}) \times 100$.

118 2.7 Protein Content

119 The micro Kjeldahl method was used to estimate the crude protein content of leaf powder.
120 2g of oven-dried leaf material was placed in a Kjeldahl flask to which 30ml of concentrated
121 sulfuric acid, 10g potassium sulphate and 1g copper sulphate was added. The mixture was
122 heated gently first and then strongly (once the frothing had ceased). The colorless or clear

123 solution obtained was heated for another one hour, cooled and diluted with distilled water to
 124 the mark in a 100ml volumetric flask. 10ml from the digest was measured into the
 125 decomposition chamber of the distillation apparatus and 15ml of 40% NaOH was added and
 126 the ammonia released was trapped into 20ml of 2% boric acid solution containing mixed
 127 indicator. A colour change from pink to green was observed as the ammonia being trapped.
 128 Distillation was continued for 5 minutes and the boric acid-ammonia solution so obtained
 129 was titrated against 0.1N HCl. The nitrogen content (%) was calculated by using the formula:
 130 % nitrogen = $(T-B) \times N \times 0.014 \times D \times 100 / \text{Weight of the sample} \times V$, where T is sample
 131 titration reading, B is blank titration reading, N is normality of HCl, D is dilution of sample
 132 after digestion, V is volume of sample taken for distillation and 0.014 is milliequivalent weight
 133 of nitrogen. % crude protein was calculated using the formula:

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$$\% \text{ crude protein} = 6.25 \times \% \text{ nitrogen, where } * \text{ is correction factor.}$$

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137 2.8 Total Carbohydrate Content

138 The content of total carbohydrates (%) was calculated by using the formula:

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$$\text{Carbohydrate (\%)} = 100 - (\% \text{ ash} + \% \text{ moisture} + \% \text{ fat} + \% \text{ protein})$$

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142 2.9 Energy Value

143 The nutritive value was determined by using the formula:

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$$\text{Energy value} = (4 \times \% \text{ protein}) + (9 \times \% \text{ fat}) + (4 \times \% \text{ carbohydrate})$$

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147 2.10 Mineral Composition of Leaf

148 ICP-OES technique was employed to estimate the content of major minerals (Calcium,
 149 sodium, potassium, magnesium and phosphorus) and minor minerals (chromium, copper,
 150 iron, manganese, nickel and zinc) in the powdered leaf material. In brief, 1g of powdered leaf
 151 material was digested using 10ml of ultrapure metal free nitric acid in a microwave digester,
 152 the content was filtered through Whatman filter paper No. 1 and diluted to 25 ml with distilled
 153 water. The digested leaf sample was aspirated into ICP-OES to estimate the content of
 154 major and minor elements. Instrument configuration and experimental conditions are shown
 155 in Table 1.

156
 157 **Table 1: ICP-OES conditions**

Power (kW)	1.2
Plasma flow (L/min)	15.0
Auxiliary flow (L/min)	1.50
Nebulizer flow (L/min)	0.75
Sample flow rate (L/min)	1.5
Replicate read time (s)	3.00
Instrument stabilization delay (s)	15.0
Sample uptake delay (s)	10.0
Pump rate (rpm)	15.0
Rinse time (s)	10.0
Spray chamber	Cyclonic type

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2.11 Preparation of Extract for Antimicrobial Activity

162 Maceration technique was performed for extraction of leaf of *M. stenopetala*. In brief, 10g of
163 powdered leaf material was added to 100ml methanol taken in a clean conical flask. The
164 flask was shaken well and left for two days with occasional stirrings. The content of the flask
165 was filtered through Whatman filter paper No. 1. The filtrate was evaporated to dryness and
166 used for determining antimicrobial activity [17].

167

168 **2.12 Antibacterial Activity of Leaf Extract**

169 Two Gram positive bacteria namely *Staphylococcus aureus* and *Bacillus subtilis* and three
170 Gram negative bacteria namely *Pseudomonas aeruginosa*, *Escherichia coli* and *Ralstonia*
171 *solanacearum* were used to assess their susceptibility to leaf extract. The test bacteria were
172 inoculated into sterile Mueller-Hinton (M-H) broth tubes and incubated overnight at 37°C.
173 Agar well diffusion assay was carried out to investigate antibacterial activity of leaf extract of
174 *M. stenopetala*. The M-H broth cultures of test bacteria (0.5 McFarland standard turbidity)
175 were swab inoculated on sterile M-H agar plates under aseptic conditions. With the use of
176 sterile cork borer, wells of 6mm diameter were punched in the inoculated plates. 200µl of
177 leaf extract (25 and 50mg/ml of DMSO), reference antibiotic (Chloramphenicol, 1mg/ml of
178 sterile distilled water) and DMSO were transferred into respective wells. The plates were left
179 for 30 minutes and then incubated for 24 hours at 37°C. Zones of inhibition formed around
180 the wells were measured using a ruler [18, 17].

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182 **2.13 Determination of Minimum Inhibitory Concentration (MIC)**

183 The MIC of leaf extract of *M. stenopetala* was determined by broth dilution method. The
184 extract dilutions (ranging from 50 to 0.0mg/ml) were tested against all test bacteria except *R.*
185 *solanacearum*. Two-fold dilutions of extract were prepared in sterile M-H broth tubes, the
186 tubes were inoculated with test bacteria and incubated at 37°C for 24 hours. The MIC of
187 extract was determined by observing the visible growth of the test bacteria after incubation.
188 The dilution showing no visible growth of bacteria was considered as the MIC of extract [19].

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190 **2.14 Antifungal Activity of Leaf Extract**

191 Poisoned food technique was performed to investigate the potential of leaf extract of *M.*
192 *stenopetala* to inhibit three phytopathogenic fungi namely *Colletotrichum capsici*, *Fusarium*
193 *oxysporum* f.sp. *zingiberi* and *Bipolaris sorokiniana*. In brief, the control (without extract) and
194 poisoned (0.5 and 1.0mg extract/ml of medium) Potato dextrose agar plates were inoculated
195 with test fungi by point inoculation technique aseptically followed by incubating the plates in
196 upright position at room temperature. The size (diameter, in cm) of fungal colonies in mutual
197 perpendicular directions was measured after an incubation of 72 hours. Antifungal activity
198 was calculated using the formula:

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$$200 \text{ Inhibition of mycelial growth (\%)} = (C - T / C) \times 100$$

201

202 Where C and T refers to diameter of fungal colonies on control and poisoned plates
203 respectively [17].

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205 **3. RESULTS AND DISCUSSION**

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207 **3.1 Proximate Composition of *M. stenopetala* Leaf**

208 It has been shown that the leaves of *M. stenopetala* are used traditionally as food as well as
209 medicine for treatment of various ailments [1, 5]. The result of proximate analysis of leaf of
210 *M. stenopetala* is shown in Table 2.

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Table 2: Proximate composition of *M. stenopetala* leaf

Parameter	Content
Moisture	08.10%
Ash	10.76%
Fat	05.25%
Protein	26.33%
Carbohydrates	42.20%
Crude fibre	07.34%
Energy value (Kcal/100g)	321.45

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The moisture content of leaf of *M. stenopetala* was found to be 8.10%. In the present study, the leaf of *M. stenopetala* was found to contain an appreciable amount of ash (10.76%) and the ash content indicates the mineral composition of the sample. The ash content of leaf (12.6%) and leaf meal (11.8%) of *M. stenopetala* was high as revealed by the study of Abuye et al. [1] and Melesse et al. [2] respectively.

Carbohydrates serve as the structural and storage components in cells. They are most prevalent in plant kingdom. Carbohydrates such as sugars are used as energy sources by cells. Carbohydrate supplies energy to brain and muscle cells and contributes to fat metabolism and spare proteins as an energy source [16]. In the present study, the leaf of *M. stenopetala* was found to contain an appreciable quantity of carbohydrates (42%). In an earlier study, higher carbohydrate content (51%) was observed in the leaves of *M. stenopetala* [1].

Like carbohydrates, proteins are essential macronutrients in diet. Proteins serve as source of energy and adequate amount of required amino acids. Proteins serve several roles such as enzymes, hormones, carriers and antibodies. The deficiency of proteins leads to various ill effects like growth retardation, edema, muscle wasting and collection of fluids in the body of children [20, 16]. In the present study, the content of proteins in the leaf of *M. stenopetala* was found to be 26.33%. The crude protein content of leaf meal of *M. stenopetala* was found to be 30.6% [2]. The study of Abuye et al. [1] revealed a low content of proteins (9%) in the leaves. The seeds were shown to contain a higher concentration of protein in the range 40.5 to 44.4% [3].

Estimation of crude fibre is based on treating the moisture and fat-free leaf sample with dilute acid (1.25%) and then with dilute alkali (1.25%) which is similar to the gastric and intestinal action in the process of digestion. The fibre content of food aids in digestion and absorption of water from the body and bulk stool. Fibre in food helps in softening of stool and hence, prevents constipation [20, 16]. The crude fibre content of leaf of *M. stenopetala* was found to be 7.34%. In an earlier study, Melesse et al. [2] found slightly higher content of crude fibre in leaf meal of *M. stenopetala* (8.3%). However, the study of Abuye et al. [1] showed high content of crude fibre (20.8%) in the leaves of *M. stenopetala*. The crude fibre content of seeds was found to be lesser (4.7-5.4%) as revealed by the study of Seifu [3].

The term crude fat is often used synonymously with ether extract and generally refers to free lipids that can be extracted into less polar solvents such as diethyl ether or petroleum ether. Among solvents, petroleum ether is generally used as it is low cost and is less hygroscopic and flammable than diethyl ether. In this study, the crude fat content of sample was estimated by weight difference method by extracting a known quantity of the sample with petroleum ether. The crude fat content was found to be 5.25%. The study of Abuye et al. [1] revealed a slightly higher fat content (5.8%) in the leaves of *M. stenopetala*. The content of crude fat in leaf meal of *M. stenopetala* was found to be slightly lesser (4.73%) when compared to the result of this study [2].

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259 Energy value of *M. stenopetala* leaf was found to be 321.45 Kcal/100g. The metabolizable
260 energy of leaf meal of *M. stenopetala* was found to be 299.2 Kcal/100g [2]. An energy value
261 of 290.6 Kcal in the leaf of *M. stenopetala* was observed in the study of Abuye et al. [1].
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263 **3.2 Mineral Content of *M. stenopetala* Leaf**

264 Minerals are one among the micronutrients and are inorganic substances that represent
265 comparatively smaller portion of the diet when compared to major nutrients such as proteins,
266 carbohydrates and fats. These minerals do not yield energy but are too important as they are
267 needed for several metabolic processes. On the basis of daily requirement, mineral
268 elements can be classified into major and minor elements. The deficiency of minerals leads
269 to a variety diseases or disorders in plants as well as animals. Several methods are
270 available to estimate mineral content of foods and other samples. One among those is ICP-
271 OES technique which has an advantage over other analytic techniques as the technique can
272 estimate several elements at a time. ICP-OES technique is widely used for elemental
273 determination of a large variety of specimen types including plants [16]. The quantity of
274 major and minor minerals in the leaf of *M. stenopetala* is shown in Table 3. Among minor
275 elements, the content of iron and nickel was highest and least respectively. Next to iron,
276 manganese was detected in high quantity. In case of major elements, potassium content
277 was found to be highest while the content of sodium was least. The content of calcium was
278 highest next to potassium. However, the study of Abuye et al. [1] revealed higher content of
279 calcium when compared to potassium in the leaves of *M. stenopetala*. In an earlier study, the
280 content of calcium was detected in high quantity when compared to the quantity of
281 phosphorus in leaf meal of *M. stenopetala* [2] which is similar to result of our study. The
282 study of Abuye et al. [1] showed the high content of iron in leaves when compared to zinc.
283 Similar result was obtained in our study also.
284

285 **Table 3: Content of minerals in leaf of *M. stenopetala***

Element	Content (ppm)
Manganese	101.93
Chromium	5.22
Copper	5.95
Iron	962.50
Nickel	2.89
Zinc	27.32
Potassium	18426.75
Magnesium	2158.83
Calcium	9716.03
Sodium	1519.68
Phosphorus	3022.73

287 288 **3.3 Antibacterial Activity of Leaf Extract of *M. stenopetala***

289 Interest in plants with antimicrobial activity has been tremendously increased in these years
290 due to several drawbacks such as high cost, adverse effects and emergence of resistant
291 bacterial strains that are associated with the use of antibiotics [21, 17]. The result of
292 antibacterial activity is shown in Table 4. The extract was effective in inhibiting all test
293 bacteria except *R. solanacearum*. Extract exhibited dose dependent inhibition of test
294 bacteria. Susceptibility to extract was marked in case of Gram positive bacteria when
295 compared to Gram negative bacteria. *Bacillus subtilis* and *Pseudomonas aeruginosa* were
296 inhibited to high extent among Gram positive and Gram negative bacteria respectively.

Inhibition of test bacteria by reference antibiotic was marked when compared to extract. Here also, Gram positive bacteria showed marked susceptibility. DMSO did not show inhibition of test bacteria. The observed results were also supported by MIC values. The MIC of extract, as determined by broth dilution technique, was least for *B. subtilis* and highest for *E. coli*. Extracts and purified compounds from *M. stenopetala* were shown to exhibit antibacterial activity. In an earlier study, Biffa [22] showed marked antibacterial efficacy of aqueous and methanolic extracts of leaf and bark of *M. stenopetala* against mastitis causing organisms namely *Staphylococcus aureus*, *Streptococcus agalactiae* and *Streptococcus dysagalactiae*. The study of Walter et al. [7] showed the potential of methanol and n-hexane extract of seeds of *M. stenopetala* to inhibit bacteria implicated in causing waterborne diseases. The acetone extract and the purified compounds from root wood of *M. stenopetala* exhibited marked activity against *E. coli* [23]. More recently, Chekesa and Mekonnen [8] evaluated antibacterial activity of solvent extracts of various parts of *M. stenopetala*. Seed and root bark extracts displayed inhibitory activity against test bacteria. While leaf extracts did not show any activity. However, in the present study, the leaf extract of *M. stenopetala* showed marked activity against test bacteria.

Table 4: Antibacterial activity of extract of *M. stenopetala*

Test bacteria	Zone of inhibition in cm				MIC (mg/ml)
	Extract 25mg/ml	Extract 50mg/ml	Antibiotic	DMSO	
<i>S. aureus</i>	1.7	2.2	3.6	0.0	1.53
<i>B. subtilis</i>	1.9	2.4	3.6	0.0	0.76
<i>P. aeruginosa</i>	1.4	1.7	2.8	0.0	3.06
<i>E. coli</i>	1.3	1.6	3.1	0.0	6.12
<i>R. solanacearum</i>	0.0	0.0	2.3	0.0	ND

'ND'- Not determined

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3.4 Antifungal Activity of Leaf Extract of *M. stenopetala*

The control of plant diseases caused by phytopathogenic fungi is often achieved with the use of synthetic fungicides. However, their use is associated with several drawbacks such as high cost, environmental pollution and emergence of resistant fungal strains. This triggered immense interest in searching possible alternatives for plant disease control. Extracts and metabolites from higher plants appear to be potential alternatives for chemical agents. Studies have shown the inhibitory effect of several plant species against pathogenic fungi [24, 17]. In the present study, the inhibitory effect of leaf extract against three phytopathogenic fungi was assessed by Poisoned food technique in which the reduction in the mycelial growth of test fungi in poisoned plates is considered positive for antifungal activity. The method has been extensively used for evaluating antifungal activity of various kinds of samples including plant extracts. The extract caused concentration dependent inhibition of mycelial growth of test fungi. At 0.5mg/ml concentration, only *B. sorokiniana* was inhibited to >50%, however, at concentration 1.0mg/ml, all test fungi were inhibited to >50%. Among the fungi, marked susceptibility was observed in case of *B. sorokiniana* followed by *C. capsici* and *F. oxysporum* (Table 5).

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Table 5: Antifungal activity of extract of *M. stenopetala*

Test fungi	Colony diameter in cm (% inhibition)		
	<i>C. capsici</i>	<i>F. oxysporum</i>	<i>B. sorokiniana</i>
Control	3.2	4.8	3.5
Extract 0.5mg/ml	1.8 (43.75)	2.8 (41.66)	1.2 (65.71)
Extract 1.0mg/ml	1.2 (62.50)	2.1 (56.25)	0.8 (77.14)

4. CONCLUSION

The result of proximate analysis and mineral estimation of the plant *M. stenopetala* highlighted the potential use of the plants as food and feed supplement. The result of nutritive composition of leaf justifies utilization of the leaf as food. Incorporation of leaf of *M. stenopetala* in the food of poultry and livestock may prove to be useful in terms of adequate nutrition as protein supplement. The plant can also be used to prevent and treat diseases caused by pathogenic microorganisms. It is evident from the study that the leaf contains antimicrobially active principles which are to be isolated and subjected to antimicrobial activity. The future study warrants for the isolation and characterization of the different secondary metabolites and their efficacy evaluation.

COMPETING INTERESTS

All the Authors Have Declared That No Competing Interests Exist

AUTHORS' CONTRIBUTIONS

This work was designed and planned by RHL and PKTR and experimental work was carried out in collaboration with all the authors. PKTR, DD and TS were involved in collection and antimicrobial activity of the plant material. The proximate and metal analysis was performed by VBN and RHL. The PKTR and RHL wrote the first draft of the manuscript. All authors read, critically reviewed the article and approved the final manuscript.

CONSENT

Not Applicable

ETHICAL APPROVAL

Not Applicable

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