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# Studies on bioactivities of various parts of *Murraya koeningii* (l) spreng (curry tree) on fungal isolates from tomatoes

**Ijato, J. Y.[1]*, Olajide, O. O.[2] and Ojo, B. O.[3]**

[1]Department of Plant Science and Biotechnology, Faculty of Science, Ekiti State University, P.M.B 5363, Ado-Ekiti, Ekiti State, Nigeria.
[2]Department of Crop, Horticulture and Landscape Design, Faculty of Agriculture, Ekiti State University, P.M.B 5363, Ado-Ekiti, Ekiti State, Nigeria.
[3]Department of Biology, the Polytechnic, Ibadan, Nigeria.

*Corresponding author. Email: considerureternity@gmail.com



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**ABSTRACT:** Antifungal *in vitro* effects of the aqueous extracts of different parts (leaves, stem and root) of *Murraya koenigii* were evaluated against six rot fungi of tomato fruits namely, *Aspergillus niger, Aspergillus flavus, Fusarium oxysporum, Mucor sp, Rhizoconia solani* and *Rhizopus stolonifer.* These fungi were identified based on microscopic and macroscopic characteristics. Hot aqueous extracts of the plants were obtained using standard techniques. Sensitivity test was done using agar well diffusion method; the set up was incubated at room temperature (28 ± 2°C) for 24 hours. Development of zones of inhibition on the plates were observed and measured. The results showed that the tested extracts exhibited antifungal activities against the test (rot) organisms. The therapeutic values of various concentrations of aqueous *Murraya koenigii* extracts (5, 10, 15, 20 and 25%) were evaluated against fungal rot isolates. All the extracts showed significant ($p<0.05$) reduction of mycelia growth of the fungal isolates. Higher concentration of 25% favoured higher mycelial growth reduction. Maximum percentage inhibition was observed with the leaf extracts (*F. oxysporum,* 77.91%, followed by *A. flavus*, 77.10%), followed by the stem extract (*A. flavus,* 73.80%, followed by *F. oxysporum*, 71.43%) and lastly the root extract (*A. flavus*, 71.90%, followed by *F. oxysporum*, 67.10%). The extracts similarly inhibited vegetative growth and displayed significant effects on the morphology of the fungal hyphae. *M. koeningii* as the test plant is readily available as it offers potential safe alternative for use as biocide and it could be harnessed for effective management of post-harvest tropical fruit diseases.

**Keywords:** Antifungal, fungal, *Murraya koeningii*, tomatoes.

## INTRODUCTION

*M. koenigii* belong to the family *Rutaceae* which represent more than 150 genera and 1600 species. *M. koenigii* commonly known as curry leaf is native to India, Sri Lanka, Bangladesh and the Andaman Islands. Later spread by Indian migrants, they are now grown in other parts of the world where Indian immigrants settled (Satyavathi et al., 1999). It is a highly values plant for its characteristic aroma and medicinal value. It is an important export commodity from India as it fetches good foreign revenue. A number of chemical constituents from every part of the plant have been extracted. The curry leafs are naturalized in forests and waste land throughout evergreen shrubs or small tree are up to 4 mm tall. Leaves are arranged spirally, imparipinnate with 17-31, leaflets, stipules are absent. Leaflets are alternate, ovate to ovate lanceolate or orbicular, 2-5 cm. Glandular are dotted, base obtuse to rounded and slightly asymmetrical, apex notched, margin is entire or irregularly toothed (Adewunmi, et al., 2001). Flowers are bisexual, regular aromatic, pedicel is short, carlyx are with tiny ovate teeth, and petals are oblanceolate to oblong, 5 to 7 mm long, glandular, white. Stamens are 10 in number, ovary is superior, stigma capitates, and fruit is ovoid to oblong, grandular berries.

*M. koenigii* comprises about 15 species which are

distributed from continental Asia throughout the Malaysian region to north-eastern Australia and New Caledonia. Several species are cultivated throughout the tropics. *M. koenigii* can be planted on red sandy, loam soils with good drainage as support for better leaf yield. The main period of availability of curry leaf fruits is July to August. Curry leaves are propagated mainly from ripe and fresh seeds as the seeds should be pulped and planted in nursery beds or polyethylene bags. The whole fruit can be planted, but it's best to remove the pulp before planting in potting mix that is kept moistened. Stem cutting can also be used as a means of propagation. *M. Koenigii* grows best in deep well-drained soil in full sun to partial shade; in Africa it is either kept in large pots or grown in home garden (Akobundu et al.,1998), flowering is from March to June and fruiting from June to August. After transplanting, it takes 12 to 15 months before the leaves can be harvested. One year old seedlings are suitable for planting; one seedling is planted at the centre of the pit. Immediately after planting, the pits are irrigated on the third day, the second irrigation is given once in a week (Handa, 1996). Leaves can be refrigerated in airtight containers for up to 2 weeks without loss of flavor. They can also be frozen from storage for year round use. Curry leaves have been used for centuries almost in all the parts of the world.

Herbal medicine is the study and the use of medicinal properties of plants (Lichterman, 2004). This herb possesses several medicinal qualities. For example, its leaves and bark can be used as a stimulant, tonic, stomachic, and carminative (Kirtikar and Basu, 1935). It can also assist in reducing blood sugar if these leaves are consumed early in the morning before breakfast. Curry leaves are a great source of various vitamins and minerals, and are also a good source of vitamin A and vitamin C, folic acid, niacin, thiamin, riboflavin and calcium. Each of these vitamins plays vital role in health living. Vitamin C is required for strengthening the immune system. Thiamin is known to have a role in organ and nervous system development. Other than vitamins and minerals, studies have shown that curry leaves contain antioxidants which are useful against free radical damage and oxidative stress in the body (Gupta et al., 2009). A paste of curry leaves can be prepared against diseases like diarrhea and dysentery which often affect infants and toddlers (Satyawati et al., 1999). In the subcontinent of India, except in the higher parts of the Himalayas. It is basically used as a spice and is an aromatic deciduous tree which is 5 meters tall and fifteen to forty centimeters in diameter. Curry powder made after pulverizing curry leaves, is used as flavor in stew, and a variety of soups, chutneys, breakfast dishes like upma etc. In some parts of Southeast Asia, curry leaves are chewed because they are believed to aid digestion and particularly suitable for preventing diarrhea. Its leaves are used in many dishes and for medicinal purposes in India and neighboring countries (Bhattacharya ,1998).

Maberly (1998) reported the properties *of M. koenigii* to include much value as anti-diabetic, antioxidant, antimicrobial and anti-inflammatory. In the last decade, demand for medicinal plants and its products as well as the traditional health system has attracted global interest due to growing recognition of the drugs from natural products, food supplements and flavors (Dhar et al., 2000). The essential oils and carbazole alkaloids from *M. Koenigii* show many interesting pharmacological activities, including antibacterial, antioxidant, antitumor and hypoglycaemic activities, and more research is needed to evaluate its potential (Adebaye, 1997).

A large number of studies have been carried out on the chemical composition of the volatile oil of *M. koenigii*. The leaves contain 35 to 65 compounds, mainly monoterpenes and sesquiterpenes depending on the seasonal variation, geographical location and age of the leaves, and which constitute about 95% of the essential oil (Onayade et al., 2000). Constituents of the essential oil of the leaves that can occur in high concentrations are a-copaene, b-ocimene and same minor compounds are b-pinene and b-phellandrene. *M. koenigii* is also a major source of carbozole alkaloids. From the stem bark, murrayanine, girinimbine, isomurayazoline has been isolated (Adewunmi et al., 2001). The crushed bark and roots are used externally to treat skin eruptions and bites of poisonous animals.

The fresh leaves are eaten to treat dysentery and a leaf infusion is drunk to stop vomiting. In northern Nigeria, *M. koenigii* is used traditionally as a stimulant and for management of diabetes (Palaniswamy, et al., 2003). The leaves are added top soup with crayfish to treat herpes, scurvy and post-partum pain. Young twigs are used as tooth brush, and are reported to strengthen the gums and the teeth. In Ayurvedic medicine, preparations of the leaves, bark and roots are used for enhancing blood circulation, digestion and metabolism as well as for its anti-inflammatory actions (Onayade *e*t al., 2000). The fresh or dried leaves are commonly used in flavoring vegetables but are reported to loose flavour upon drying. The essential oil (curry leaf oil), obtained from the leaves by distillation is used in the production of soap (Patel et al., 2009).

African trade of leaves for medicinal or culinary use is probably very limited, except for Mauritius and reunion. In Asia, the root wood is locally considered the best of all woods for making small objects. As the supplies are often extremely limited, the wood fetches high prices and is sold by the piece, particularly in the rural areas where they are used as first treatment for sick people (Kirtikar and Basu, 1935). Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions, and to defend against attack from predators such as insects, fungi and herbivorous mammals (Stepp, 2004). Many of these plants have beneficial effects on long term health when consumed by humans, and can be used to effectively treat human diseases (Sumner, 2000).

Natural products or plants derived compound contribute

to a great extent in the fight against pathogenic micro-organisms (Vyvyan, 2002). The biological inhibitions by different natural substances, such as essential oils and plant extracts have been investigated widely against fungal activities. Singh et al. (1998) determined fungi toxicity of extracts from 11 higher plants against a range of fungi based on sugarcane pathogens. So, it is vital to investigate the antifungal effects of curry leave (*M. koenigii*) plant extracts using agar well diffusion method, investigating extract types on fungal rot pathogens of tomatoes namely: *Aspergillus niger, A. flavus, Fusarium oxysporum, Mucor sp, Rhizoconia solani* and *R. stolonifer* with the purpose of mitigating post-harvest rot of tomatoes and to extend tomato fruits shelf life.

## MATERIALS AND METHOD

### Collection of samples

Samples of infected and healthy tomatoes (L*ycopersicum esculentum*) fruits were collected from neighborhood market; these were placed in sterile polyethylene bags and were taken to the laboratory for further studies. The samples were sterilized using 70% alcohol to eliminate surface contaminants. The samples were cultured using direct plating method where sterile inoculating needle was used to cut a piece from each of the tomato fruits from a portion of advancement of the rot and carefully placed on the media in separate petri dishes for each sample and the inoculating needle was sterilized using spirit lamp after each use. The cultures were incubated at room temperature (28 ± 2°C) for five days to develop the colony.

### Isolation of rot pathogens from spoilt tomato fruits

Tomatoes were washed in running tap and then surface sterilized with 1% sodium hypochlorite solution (NaOCl) for 3 minutes (Suleiman, 2011). These were then, rinsed in changes of sterile distilled water (SDW) and this was dried on sterile tissue paper. Pieces of rotten and tomatoes fruits at the advance margin of rot and unwritten portion were placed in a sterile soft tissue paper and were plated on potato dextrose agar (PDA) and Sabouraud dextrose agar (SDA) in the Petri dishes, and observed for fungal growth 3 days after plating. Pure cultures of the isolates were obtained by sub-culturing each discrete colony into freshly prepared PDA and SDA in sterile Petri dishes. Each fungal isolate was plated at the centre of the Petri dishes.

### Identification of fungal isolates: Macroscopic and microscopic characterization

Macroscopic characterization such as colour of the colony, texture, topography, shape, and diameter of the colony were employed to identify the isolates while microscopic characterization such the shape, size, and the colour of the conidiosphore, mycelium, conidia, sporangiosphore, and vesicles were all studied. To accomplish this, a small portion of achieve fungal growth from the Petri dish was mounted in a drop of lactophenol (cotton blue) on a clean and sterile glass slide and studied under the microscope (Watanebe, 2010, Philips, 2013, Valencia et al., 2019). Characters were compared with already identified species with the help of identification key by Klish (2002) and Larone (2002).

### Collection and preparation of curry plant extracts

Samples of fresh parts (leaves, stem, bark and root) of *Murraya koeningii* were collected from a vegetation site in Ado Ekiti. The identity of the collected plant parts was authenticated by the herbarium unit of Ekiti State University, Ado Ekiti. The plant parts were removed, washed separately in several changes of water to removed dirt and rinsed in sterile distilled water, wiped, clean before surface sterilizing using 70% alcohol (Kumar et al., 2013) after which they were shade dried over a paper towel on laboratory bench at room temperature (28 ± 2°C). Thereafter, the various plan parts were pulverized into fine powder, thirty grams of each powder sample of various parts of the test plant was then placed in a porous cellulose thimble. The thimble was placed in an extraction chamber which was suspended above the flask containing the solvent and below a condensed. The flask was heated and the solvent evaporated and moved up into the condenser where it was into liquid and tricked into the chamber containing the sample. The liquid continues to accumulate in the extractor containing the sample until it reached a point and overflowed and trickled back into the boiling flask. After the extraction protocols, the flask containing the extracted ingredient was removed and the solvent removed using rotary evaporator, to obtain the active ingredient in a paste form and different concentrations viz: 5,10, 15, 20 and 25% aqueous extracts were prepared by dilution method from the stock solution.

### Effect of plant extracts on fungal growth

Pour plate method was used to determine antimicrobial property of the test plant. Molten nutrient agar was dispensed into sterile Petri dishes and this was allowed to cool down to 45°C and the bacterial inoculum was streaked on the medium. Wells were punched into the agar using 4 mm cork borer and the wells were filled with 1 ml of the test plant extracts. The plates were incubated at 37°C for 24 hours. The antibacterial activity of the test plant was determined by measuring the diameter of the zone of inhibition using metre rule. Distilled water was taken as control.

**Table 1.** Percentage inhibition of mycelial growth of fungal isolates from tomato fruits with different concentrations of cold aqueous leaf extract of *M. koenigii.*

| Concentrations of extracts % | Fungal isolates | | | | | |
|---|---|---|---|---|---|---|
| | ***A. niger*** | ***A. flavus*** | ***F. oxysporum*** | ***Mucor sp.*** | ***R. solani*** | ***R. solonifer*** |
| Control | 00.00$^{d}$ | 00.00$^{c}$ | 00.00$^{d}$ | 00.00$^{c}$ | 00.00$^{d}$ | 00.00$^{d}$ |
| 5 | 37.50$^{c}$ | 59.33$^{a}$ | 53.81$^{b}$ | 36.11$^{b}$ | 40.40$^{c}$ | 18.70$^{c}$ |
| 10 | 50.00$^{b}$ | 67.60$^{a}$ | 64.29$^{b}$ | 46.67$^{b}$ | 53.30$^{b}$ | 18.75$^{c}$ |
| 15 | 52.92$^{b}$ | 71.40$^{a}$ | 68.57$^{a}$ | 50.56$^{b}$ | 57.10$^{b}$ | 25.00$^{b}$ |
| 20 | 65.00$^{a}$ | 72.80$^{b}$ | 71.43$^{a}$ | 51,67$^{a}$ | 67.10$^{a}$ | 31.25$^{a}$ |
| 25 | 76.67$^{a}$ | 77.10$^{a}$ | 77.91$^{a}$ | 55.10$^{a}$ | 71.40$^{a}$ | 36.20$^{a}$ |

Values followed by the same letter are not significantly different at ($p<0.05$) according to Duncan multiple range test.

**Table 2.** Percentage inhibition of mycelial growth of fungal isolates from tomato fruits with different concentrations of cold aqueous stem extract of *M. koenigii.*

| Concentrations of extracts % | Fungal isolates | | | | | |
|---|---|---|---|---|---|---|
| | ***A. niger*** | ***A. flavus*** | ***F. oxysporum*** | ***Mucor sp.*** | ***R. solani*** | ***R. solonifer*** |
| Control | 00.00$^{c}$ | 00.00$^{d}$ | 00.00$^{d}$ | 00.00$^{c}$ | 00.00$^{d}$ | 00.00$^{c}$ |
| 5 | 32.50$^{b}$ | 53.60$^{c}$ | 50.95$^{c}$ | 32.20$^{b}$ | 32.30$^{c}$ | 12.50$^{b}$ |
| 10 | 30.00$^{b}$ | 60.30$^{b}$ | 56.19$^{b}$ | 46.60$^{a}$ | 43.80$^{b}$ | 12.50$^{b}$ |
| 15 | 37.50$^{a}$ | 62.19$^{a}$ | 65.70$^{a}$ | 47.20$^{a}$ | 50.00$^{b}$ | 15.00$^{b}$ |
| 20 | 37.50$^{a}$ | 70.00$^{a}$ | 69.05$^{a}$ | 49.40$^{a}$ | 62.80$^{a}$ | 20.00$^{a}$ |
| 25 | 47.50$^{a}$ | 73.80$^{a}$ | 71.43$^{a}$ | 50.00$^{a}$ | 68.50$^{a}$ | 23.70$^{a}$ |

Values followed by the same letter are not significantly different at ($p < 0.05$) according to Duncan multiple range test.

**Percentage inhibition of mycelia growth** (**mycelia extension of fungi)**

The method of Amadioha and Obi (1999) was used to determine the effect of extracts on mycelia extension of the fungi obtained by placing one disc (3 mm diameter) of 5 days old culture of the pathogens in each of five Petri dishes (1 cm diameter) with 170 ml PDA medium and 3 ml leaf extract. The control experiments were setup with 3 ml of sterile distilled water. Five replications of leaf extracts agar per isolate were incubated at room temperatures (28 $\pm$ 2°C) for 7 days. Daily measurements of the mycelia extension of the cultures were determined by measuring culture along two diameters mycelia growth inhibition was taken as growth of the fungus on the leaf extract agar expressed as percentage of growth on the PDA. Fungitoxicity was determined in form of percentage growth of colony inhibition and calculated according to this formula:

$$\text{Growth inhibition } (\%) = 1 + \frac{\text{DC} - \text{DT}}{\text{DC}}\ x100$$

Where DC = Average diameter of colony with control and DT = Average diameter of colony with treatment.

**Data analysis**

Data obtained from this study were subjected to analysis of variance (ANOVA) using SPSS, means were separated using Duncan multiple range test (DMRT) at 5% level of significance ($p\leq0.05$).

## RESULTS

Table 1 showed the effects of aqueous leaf extract of *Murraya koeningii* on mycelial growth of fungal isolates from tomato fruit. All the concentrations of leaf extract of *M. koenigii* exhibited significantly on mycelial growth reduction on the fungal isolates than the control where there was no inhibition. Higher concentrations supported higher inhibition as 25% concentrations was found to be the most potent against *Fusarium oxysporum* (77.91%) followed by *Aspergillus flavus* (77.10%) while the least inhibited organism was *Rhizopus stolonifer* (36.20%).

Table 2 showed that all the concentrations of stem extract of *M. koenigii* exhibited significant inhibitory effect on mycelial growth of all the fungal isolates than the control where there was no inhibition. Higher concentrations of stem extract of *M. koenigii* supported higher inhibition as 25% concentrations were found to be the most inhibitive against *A. flavus* (73.80%) followed by *F. oxysporum* (71.43%) while the least inhibited organism was *R. stolonifer* (23.70%).

Table 3 showed that all the concentrations of stem extract of *M. koenigii* exhibited significant inhibitory effect on mycelia growth of all the fungal isolates than the control

**Table 3.** Percentage inhibition of mycelial growth of fungal isolates from tomato fruits with different concentrations of cold aqueous root extract of *M. koenigii.*

| Concentration of extracts (%) | Fungal isolates | | | | | |
|---|---|---|---|---|---|---|
| | ***A. niger*** | ***A. flavus*** | ***F. oxysporum*** | ***Mucor sp.*** | ***R. solani*** | ***R. solonifer*** |
| Control | 00.00$^{c}$ | 00.00$^{d}$ | 00.00$^{d}$ | 00.00$^{c}$ | 00.00$^{d}$ | 00.00$^{d}$ |
| 5 | 25.00$^{b}$ | 44.70$^{c}$ | 42.80$^{c}$ | 16.60$^{b}$ | 30.00$^{b}$ | 62.50$^{c}$ |
| 10 | 25.00$^{b}$ | 51.40$^{b}$ | 51.90$^{b}$ | 25.50$^{b}$ | 30.00$^{b}$ | 87.50$^{c}$ |
| 15 | 37.00$^{a}$ | 61.90$^{b}$ | 62.80$^{a}$ | 33.30$^{b}$ | 49.00$^{b}$ | 12.50$^{b}$ |
| 20 | 37.90$^{a}$ | 63.80$^{a}$ | 66.10$^{a}$ | 36.60$^{b}$ | 57.10$^{a}$ | 17.50$^{a}$ |
| 25 | 43.70$^{a}$ | 71.90$^{a}$ | 67.10$^{a}$ | 45.00$^{a}$ | 61.40$^{a}$ | 21.20$^{a}$ |

Values followed by the same letter are not significantly different at ($p<0.05$) according to Duncan multiple range test.

where there was no inhibition. Higher concentrations of root extract of *M. koenigii* supported higher inhibition as 25% concentrations was found to be the most inhibitive against *A. flavus* (71.90%) followed by *F. oxysporum* (67.10%) while the least inhibited organism was *R. stolonifer* (21.20%).

## DISCUSSION

In this study, fungicidal effects of aqueous extracts of parts of *Murraya koeningii* and their respective concentrations against four fungal species viz*; Aspergillus niger, Aspergillus flavus, Fusarium oxysporum, Mucor sp, Rhizoconia solani* and *Rhizopus stolonifer* were bio assayed. *M. koenigii* was selected for his study based on the traditional usage and previous studies that have demonstrated anti-mycelial properties using various extracts (Disegha and Izionworu, 2014). Fungicidal effects of *M. koenigii* were found to be profound against *A. niger* and *F. oxysporum* in this study, this agreed with the findings of Hariom et al. (2017) who inhibited the growth of some fungi with *M. koenigii* extract effectively.

The evaluated extracts of various parts of *M. koenigii* displayed *in vitro* fungicidal effects with various values on the rot pathogens of tomatoes. Inhibitory effects of the parts of the test plant were in order of leaf, stem and lastly root extract. Higher concentration of the test plant (25%) supported higher mycelial growth reduction. Maximum percentage inhibitory effect was noticed with the leaf extracts of *M. koenigii* (*F. oxysporum*, 77.91% closely followed by *A. flavus*, 77.10%), followed by the stem extract (*A. flavus,* 73.80% followed by *F. oxysporum*, 71.43%) and lastly the root extract (*A. flavus*, 71.90% followed by *F. oxysporum*, 67.10%). The extracts similarly reduced the vegetative growth of the isolated fungi as they exhibited significant effects on the morphology of the fungal hyphae. The cold aqueous extracts of 20% concentration demonstrated the least activity against *R. solonifer.* This could be as a result of pulverized plant materials placed in cold water, some hydrolases and phenolases could have been discharged, thereby exhibiting modulatory capacity on the bioactive principles in the extracts. It could as well be related to partial extraction of the active principles (El-Mahmood et al., 2008).

Antimicrobial effects of *M. koenigii* have been reported by Sowjanya and Manohra (2012), Mohana et al. (2011), Bertha et al. (2012) and Muthumani et al. (2010). Comparison of the growth inhibition of various parts of the test plant extracts and their attendant concentrations could also be noticed. These results showed that fungicidal values of different extracts from the test plant parts were promoted as the concentration of the extracts, therefore, the bioactive capacity of the extracts was based on concentration.

The results obtained in this study is in tandem with that of Pinelo et al. (2004), who opined that chemical property of the solvent, mode of extraction as well as various compositional and structural parts of the natural products in each solvent indicates diverse reaction. In addition, the antifungal activity of plant extracts might not be due to the action of a single active compound, but the synergistic effects of several compounds that are in minor proportion in a plant as reported by Disegha and Izionworu (2014). Results from this finding indicated the potential usefulness of extracts from various parts of *M. koenigii* (curry leaf) as they displayed fungicidal effects on *A. niger, A. flavus, F. oxysporum, Mucor sp, R. solani* and *R. solonifer. M. koenigii* (curry leaf) becomes a veritable botanical for discovery of novel bioactive compounds for combating phytopathogens for the purpose of ensuring food security for teeming populace.

## CONFLICT OF INTEREST

The authors declare that they have no conflict of interests.

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