**IJPSR (2012), Vol. 3, Issue 07** **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 12 March, 2012; received in revised form 01 June, 2012; accepted 22 June, 2012

# EVALUATION OF *MURRAYA* EXTRACTS FOR CONTROLLING FUNGI GROWTH ON *ZAPOTA* THROUGH ESTIMATION STUDIES

Bertha Fernandes, Lourdes Pereira, Tricia Pereira and Shruthi SD*

P.G. Department of Biotechnology, The Oxford College of Science, Bangalore- 560 102, Karnataka, India

**Keywords**:
alcohol,
antifungal,
*Murraya*,
proteins,
sugars,
*Zapota*

**Correspondence to Author:**

**Shruthi SD**

Assistant Professor, P.G. Department of Biotechnology, The Oxford College of Science, Bangalore- 560 102, Karnataka, India

## ABSTRACT

Medicinal plants are various plants which are used in herbalism having medicinal properties. Few plants or their phytochemical constituents have been proven to have medicinal effects by rigorous science or have been approved by regulatory agencies. Shade dried leaves of *Murraya koenigii* was extracted using successive solvent extraction method using ethanol, chloroform and aqueous. Qualitative phytochemical screening showed the presence of alkaloids, phytosterols, deoxysugars, phenols, tannins, flavonoid, glycosides and anthraquinone. The extracts were tested for its antifungal activity against *Penicillium*, *Aspergillus niger, Aspergillus flavus* and *Fusarium*. *Manilkara zapota* was inoculated with the fungus and the ability of extracts to maintain the protein and sugar content was evaluated. Aqueous extract was found to be more active against *Penicillium* and *A. niger* in maintaining the protein and sugar content. Ethanol and chloroform extracts was found to be more active against *A. flavus* in maintaining the sugar content and *A. niger* in maintaining the protein content.

**INTRODUCTION:** The Indian plant *Murraya koenigii* belong to family Rutaceae, commonly called "curry leaf" in English and locally known as Karibevu [1]. The species is native of India and is found everywhere in India [2]. It commonly occurs in foothills of Himalaya, Assam, Sikkim, Kerala, Tamil Nadu, Andhra Pradesh, Maharashtra, Karnataka etc [2]. The leaves are pinnate, with 11-21cm broad; flowers are small, white and fragrant [3].

On phytochemical investigation researchers have claimed that the leaves of *Murraya koenigii* found to contain alkaloids, volatile oil, gycozoline, xanthotoxine and sesquiterpione [4]. The leaf has been found to show antioxidant activity [5], hypoglycemic activity [6], antibacterial activity [1], anti-dysentery [7] and also act as an hepatoprotective agent [3].

*Manilkara zapota* (L.) P. Royen, found abundant in India is a small to medium evergreen tree of slow growth. An infusion of the young fruits and the flowers is drunk to relieve pulmonary complaints. Sapodilla fruit regulates the metabolism and keeps the digestive tract clean. It also helps in regulating the secretion of gastric enzymes, thereby regulating metabolism. It is rich in minerals which help in formation of essential enzymes and gastric juices.

Among its many benefits related to the digestive system, weight loss and obesity prevention can also be included in the benefits. This plant has antioxidative property [8] and its fruit is preventive against biliousness and attacks of fever whereas seeds are diuretic [9]. The major constituents isolated from fruits of *Manilkara zapota* are polyphenols (methyl chlorogenate, dihydromyricetin, quercitrin, myricitrin, (+)-catechin,

(-)-epicatechin, (+)-gallocatechin, and gallic acid [10]. Because of the tannin content, young fruits are boiled and the decoction taken to stop diarrhoea.

The present study was conducted to evaluate the antifungal activity of aqueous, ethanol and chloroform extracts of leaves of *Murraya koenigii* against various fungi grown on *Manilkara zapota* through estimation of alcohol, sugar and protein.

**MATERIALS AND METHODS:**

**Collection of Plant Material:** Fresh leaves of *Murraya Koenigii* were collected in and around the Oxford College campus, HSR layout, Bangalore, Karnataka (South India) in February 2011. The taxonomic identification of the plant was confirmed and processed for further investigations.

**Collection of Test Organisms and Sample:** Test organisms *Aspergillus niger, Aspergillus flavus, Fusarium* and *Penicillium chrysogenum* were obtained from Department of Microbiology, The Oxford College of Science, Bangalore and sub cultured in Potato Dextrose Agar selective media. The fresh fruits of *Manilkara zapota* (Chickoo) procured from market was used as sample.

**Extraction of Plant Material:** Freshly collected leaves of *Murraya koenigii* were shade dried and powdered using a mechanical grinder. 4gms of pulverized leaf material were soaked in 100ml of different solvents like aqueous, ethanol and chloroform (LR grade, Merck, India) at room temperature for 48 h. Each extract was filtered under vacuum through Whatmann No. 42 (125mm) filter paper and the process repeated until all the soluble compounds have been extracted. The extracts were evaporated to dryness. The percentage yield of extracts ranged from 7-19% (w/w).

**Qualitative Phytochemical Examination of the Extracts:** The tests were done to find the presence of the active chemical constituents such as carbohydrates, alkaloids, phytosterols, glycosides, anthroquinone, saponins, phenolics, tannins, flavonoids, protein, total sugar and alcohol by the following procedure. Aqueous, ethanol and chloroform extracts were dissolved individually in 3ml of DMSO and filtered. The filtrates were used for the test [11, 12].

**Carbohydrates:** The presence of carbohydrates was determined by Benedict's method. 1ml of filtrate was taken in a clean dry test tube and 1ml of Benedict's reagent was added to it and placed in hot water for 15 mins. Formation of orange colour indicates the presence of reducing sugar.

**Alkaloids:** Alkaloids are basic nitrogenous compounds with definite physiological and pharmacological activity. Alkaloid solution produces white yellowish precipitate when a few drops of Mayer's reagent is added [13]. 1ml of Mayer's reagent was taken in a test tube containing 1 ml of filtrate. Formation of yellow colour precipitate indicated presence of alkaloids.

**Phytosterols:** Phytosterols are a group of steroid alcohols, phytochemicals naturally occurring in plants. The presence of phytosterols can be shown by Salkowski's test in which 1ml of filtrate was taken in clean test tube and conc.$H_2SO_4$ is added, thoroughly shaken and allowed to stand. Lower layer turning red indicates presence of sterols and turning yellow indicates the presence of terpenes.

**Glycosides:** Glycosides are compounds which upon hydrolysis give rise to one or more sugars (glycones) and a compound which is not a sugar (aglycone or genine). To the extract in glacial acetic acid, few drops of ferric chloride and conc. $H_2SO_4$ were added, and observed for a reddish brown coloration at the junction of two layers and the bluish green color in the upper layer [13].

**Phenolics and Tannins:** Tannin is an astringent, bitter plant polyphenolic compound that binds to and precipitates proteins and various other organic compounds including amino acids and alkaloids. Tannins are incompatible with alkalis, gelatin, heavy metals, iron, lime water, metallic salts, strong oxidizing agents and zinc sulfate, since they form complexes and precipitate in aqueous solution [14].

To 0.5 ml of extract solution 1 ml of water and 1 - 2 drops of ferric chloride solution was added. Blue color was observed for Gallic tannins and blackish green for catecholic tannins [15]. The presence of phenolics was indicated by formation of white precipitate when few drops of $FeCl_3$ solution were added to 1 ml of extract.

**Saponins:** Saponins are a class of phytochemical compounds. More specifically, they are amphipathic glycosides grouped in terms of phenomenology by the soap-like foam they produce when shaken in solutions and in terms of structure by their composition of one or more hydrophilic glycoside moieties combined with a lipophilic triterpene derivative [16]. To the extract 20 ml of distilled water was added and shaken in a graduated cylinder for 15 minutes. Formation of foam indicated presence of saponins.

**Flavonoids:** 4ml of extract solution was treated with 1.5 ml of 50% methanol solution. The solution was warmed and metal magnesium was added. To this solution, 5 - 6 drops of concentrated hydrochloric acid was added and red color was observed for flavonoids and orange color for flavones [13].

**Estimation of proteins in sample:** Different aliquots (0.2ml-1.0ml) of standard protein was pipetted out into different test tubes and made upto 1ml using distilled water. 1ml of the unknown sample was taken. The volume in all the tubes was made to 1ml using distilled water. 5ml of alkaline copper reagent was added in all tubes and incubated for 10 minutes at room temperature. Then 0.6ml of FC reagent was added and the tubes were mixed thoroughly, kept at room temperature for 30 minutes and the absorbance was read at 660nm. The same procedure was followed to estimate proteins after infection with fungus and treatment with extracts [17].

**Estimation of total sugar in sample:** Different aliquots (0.2ml-1.0ml) of standard glucose were pipetted out into different test tubes made upto 1ml using distilled water. 1ml of sample was taken as unknown. The volume was made to 1ml using distilled water; 4ml of Anthrone's reagent was added to all tubes and kept in boiling water bath for 10 minutes. Samples were cooled and the optical density was measured at 620nm using a suitable blank. The same procedure was followed to estimate sugars after infection with fungus and treatment with extracts [18].

**Estimation of alcohol in sample:** Alcohol is one of the products produced during fermentation in fruits. The percentage of alcohol can be determined by specific gravity method [19]. Density of a substance is the ratio of mass and unit volume expressed as grams/ml. The specific gravity of the liquid was then determined by finding the ratio of the liquid and the weight of the distilled water at the same temperature in a specific gravity bottle. Initially the weight of the empty specific gravity bottle was found out followed by the weight of the bottle with the sample and later weight of the bottle with distilled water. The specific gravity of alcohol was then calculated using the given formula:

$$\frac{W_2-W_1}{W_3-W_1} \times \text{Density of water at Specific Gravity}$$

W1- weight of empty specific gravity bottle

W2- weight of bottle with sample

W3- weight of bottle with distilled water

Percentage of alcohol was read against the corresponding value of specific gravity association of analytical chemistry chart (AOAC).

**RESULTS AND DISCUSSION:** The fungal samples chosen for the study were *Penicillium chrysogenum, Aspergillus niger, Aspergillus flavus* and *Fusarium.* **Table 1** shows the results of protein estimation from the samples untreated, treated with fungus and with extracts. FC method was used for the determination of protein. Initially the protein present in the ripe fruit (untreated with fungus and extract) was found out. The protein content was then determined in the fruits treated with fungus and extract after an incubation period of 7 days. A decrease in the protein content was seen in all the samples.

The ethanol and water extract sample showed a decrease in protein content compared to that of the untreated sample but more compared to the other treated (extract and fungus) samples. Chloroform extract treated samples showed lesser protein content post infection with *Aspergillus flavus, Fusarium* and *Penicillium chrysogenum.*

**Table 2** shows the results of estimation of total sugars in the samples untreated, treated with fungus and with extracts. Initially the sugar content found in the sample (untreated) fruit was high. The sugar concentration was decreased in all samples treated (fungus and extract) as well as untreated (treated only with fungus).

**TABLE 1: TEST RESULTS FOR THE ESTIMATION OF PROTEINS**

| Sl. No. | Test samples | OD at 660 nm | Conc (µg/ml) |
|---|---|---|---|
| 1 | Sample | 0.21 ± 0.1 | 350 |
| 2 | Sample + water | 0.18 ± 0.12 | 300 |
| 3 | Sample +DMSO | 0.07 ± 0.14 | 100 |
| 4 | Sample + *Penicillium* | 0.22 ± 0.20 | 320 |
| 5 | Sample + *A. niger* | 0.16 ± 0.13 | 230 |
| 6 | Sample + *A. flavus* | 0.24 ± 0.14 | 350 |
| 7 | Sample + *Fusarium* | 0.23 ± 0.15 | 330 |
| 8 | Sample+ AE + *Penicillium* | 0.22 ± 0.09 | 320 |
| 9 | Sample + AE + *A. niger* | 0.23 ± 0.12 | 330 |
| 10 | Sample + AE + *A. flavus* | 0.18 ± 0.13 | 260 |
| 11 | Sample + AE + *Fusarium* | 0.16 ± 0.15 | 230 |
| 12 | Sample + EE + *Penicillium* | 0.18 ± 0.12 | 260 |
| 13 | Sample + EE + *A. niger* | 0.24 ± 0.09 | 350 |
| 14 | Sample + EE + *A. flavus* | 0.22 ± 0.08 | 320 |
| 15 | Sample + EE + *Fusarium* | 0.14 ± 0.1 | 200 |
| 16 | Sample + CE + *Penicillium* | 0.07 ± 0.09 | 100 |
| 17 | Sample + CE + *A. niger* | 0.23 ± 0.12 | 330 |
| 18 | Sample + CE + *A. flavus* | 0.15 ± 0.11 | 220 |
| 19 | Sample + CE + *Fusarium* | 0.11 ± 0.13 | 160 |

AE- Aqueous extract, EE- Ethanol extract, CE- Chloroform extract, The values are expressed in mean ± SE, n=3.

**TABLE 2: TEST RESULTS FOR THE ESTIMATION OF TOTAL SUGARS**

| Sl. No. | Test samples | OD at 660 nm | Conc (µg/ml) |
|---|---|---|---|
| 1 | Sample | 1.80 ± 0.13 | 141 |
| 2 | Sample + water | 1.72 ± 0.12 | 135 |
| 3 | Sample + DMSO | 1.47 ± 0.09 | 114 |
| 4 | Sample + *Penicillium* | 1.25 ± 0.1 | 96 |
| 5 | Sample + *A. niger* | 1.82 ± 0.11 | 143 |
| 6 | Sample + *A. flavus* | 1.61 ± 0.13 | 126 |
| 7 | Sample + *Fusarium* | 1.68 ± 0.12 | 131 |
| 8 | Sample+ AE + *Penicillium* | 1.70 ± 0.09 | 133 |
| 9 | Sample + AE + *A. niger* | 1.79 ± 0.12 | 140 |
| 10 | Sample + AE + *A. flavus* | 1.14 ± 0.13 | 89 |
| 11 | Sample + AE + *Fusarium* | 1.82 ± 0.14 | 143 |
| 12 | Sample + EE + *Penicillium* | 1.72 ± 0.15 | 135 |
| 13 | Sample + EE + *A. niger* | 1.80 ± 0.09 | 141 |
| 14 | Sample + EE + *A. flavus* | 1.48 ± 0.11 | 115 |
| 15 | Sample + EE + *Fusarium* | 1.10 ± 0.14 | 77 |
| 16 | Sample+ CE + *Penicillium* | 1.17 ± 0.15 | 91 |
| 17 | Sample+ CE + *A. niger* | 1.69 ± 0.12 | 132 |
| 18 | Sample+ CE + *A. flavus* | 1.76 ± 0.13 | 138 |
| 19 | Sample+ CE + *Fusarium* | 1.30 ± 0.09 | 101 |

AE- Aqueous extract, EE- Ethanol extract, CE- Chloroform extract, The values are expressed in mean ± SE, n=3.

A qualitative phytochemical analysis was performed for the detection of alkaloids, glycosides, phenols, flavonoids, tannins and deoxysugars. Results of which are shown in **Table 3**.

The present study carried out on the plant revealed the presence of various medicinally important active constituents. The aqueous extract showed positive results for carbohydrates and anthroquinone. The leaf extract obtained using ethanol showed positive results for tannins, flavonoids, glycosides, anthroquinone, alkaloids, phytosterols, deoxysugars and phenols. Whereas, chloroform extract showed positive results for carbohydrates, deoxysugars, phenols, glycosides and anthroquinone.

Best result with the ethanol extract may be attributed to the reason that its stronger extraction capacity could have extracted a greater number of constituents. No alcohol content was found in the fresh, untreated

(only fungus) sample as well as the treated (extract and fungus) samples. Application of the plant extract *Murraya koenigii* on *Manilkara zapota* showed to slow down the fungal growth. *Manilkara zapota* was used as a test subject because it is easily susceptible to fungal attack. Estimation of protein content in most of the samples after the incubation period showed a decrease in the protein content. The concentration of protein remained almost the same when treated with aqueous, ethanol and chloroform extracts along the fungi namely *P. chrysogenum*, *A. niger, A. flavus* thus showing the effectivity of the plant extract as an anti-fungal agent. Sugar content was also decreased in most samples but maintained the concentration with regard to the fresh fruit sample when treated with aqueous, ethanol and chloroform along with the fungi namely *P. chrysogenum, A. niger* and *A. flavus* thus showing its effectivity. Alcohol was found to be totally absent which supports the fact that fungus as well as extracts has no role to play with its content.

**TABLE 3: PHYTOCHEMICAL ANALYSIS OF *MURRAYA KOENIGII***

| Secondary metabolites | Extracts | | |
|---|---|---|---|
| | Aqueous | Ethanol | Chloroform |
| Carbohydrates | + | _ | + |
| Alkaloids | _ | + | _ |
| Phytosterols | _ | + | _ |
| Deoxy sugars | _ | + | + |
| Phenols | _ | + | + |
| Tannins | _ | + | _ |
| Flavonoids | _ | + | _ |
| Glycosides | _ | + | + |
| Anthroquinone | + | + | _ |

'**+**' - present, '**-**'- absent

**CONCLUSION:** Plants have evolved the ability to synthesize chemical compounds that help them defend against a variety of bacteria and fungi. The plant extracts of *Murraya koeinigii* has proved to be antifungal against *A. flavus* and *Fusarium*. The results of phytochemical analysis comprehensively validate the presence of therapeutically important and valuable secondary metabolites. Along with it confirms the ethanobotanical claim of the plant as an efficient traditional medicine for fungal diseases. Further study aiming towards investigation and isolation of that particular antifungal compound is in progress.

## REFERENCES:


1. Zachariah SM, Muthumani P and Ramaseshu K: Phytochemistry and Antimicrobial screening of stem bark of *Murraya koenigii* (Linn) spreng. The Internet Journal of Pharmacology 2009; 6(2).
2. Bhattacharjee SK: Hand Book of Medicinal Plant. 3rd, pointer publishers, Jaipur, 2001.
3. Wilking JE, Mathias JK, Das K, Nidhaya ISR and Sudhakar G: Comparative Hepatoprotective Activity of Leaf Extracts of *Murraya koenigii* from Indian Subtropics. Indian Journal of Natural Product 2006; 23: 13-17.
4. Pande MS, Gupta SPBN and Pathak A: Hepatoprotective Activity of *Murraya koenigii* Linn Bark. Journal of Herbal Medicine and Toxicology 2009; 3(1).
5. Tachibana Y, Kikuzaki H, Lajis NH and Nakatani N: Antioxidative activity of Carbazoles from *Murraya koenigi* leaves. Journal of Agriculture and Food Chemistry 2001; 49(11): 5589.
6. Kesari AN, Gupta RK and Watal G: Hypoglycemic effect of *Murraya Koenigii* on normal and alloxan-diabetic rabbits. Journal of Ethnopharmacology 2005; 97(2): 247-251.
7. Adebajo AC, Ayoola OF, Iwalewa EO, Akindahunsi AA, Omisore NO, Adewunmi CO and Adenowo TK: Anti-trichomonal, biochemical and toxicological activities of methanolic extract and some carbazole alkaloids isolated from the leaves of *Murraya koenigii* growing in Nigeria. phytomedicine 2006; 13(4): 246.
8. Shui G, Wong SP and Leong LP: Characterization of antioxidants and change of antioxidant levels during storage of *Manilkara zapota* L. Journal of Agriculture and Food Chemistry 2004; 52(26): 7834-7841.
9. Ghani A: Medicinal Plants of Bangladesh with chemical constituents and uses. 2nd edn, Asiatic Society of Bangladesh, Dhaka, 2003.
10. Ma J, Luo XD, Protiva P, Yang H, Ma C, Basile MJ, Weinstein IB and Kennelly EJ: Bioactive novel polyphenols from the fruit of *Manilkara zapota* L. (Sapodilla). Journal of Natural Products 2003; 66(7): 983-986.
11. Krishnaiah D, Devi T, Bono A and Sarabatty R: Studies on phytochemical constituents of six Malaysian medicinal plants. Journal of Medicinal Plants Research 2009; 3(2): 67-72.
12. Harborne JB: Phytochemical methods. A guide to modern technique of plant analysis. Chapman and Hill, London, 1992.
13. Siddiqui AA and Ali M: Practical Pharmaceutical chemistry. 1st ed, CBS Publishers and Distributors, New Delhi, 1997.
14. McGee Harold: On food and cooking: the science and lore of the kitchen. New York, Scribner, 2004.
15. Iyengar MA: Study of Crude Drugs. 8th ed, Manipal Power Press, Manipal, India, 1995.
16. Hostettmann K and Marston A: Saponins. Cambridge: Cambridge University Press, 1995.
17. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ: Protein measurement with the Folin phenol reagent. Journal of Biological Chemistry 1951; 193: 265.
18. Hedge JE and Hofreiter BT: In: Carbohydrate Chemistry. 17th Eds. Whistler RL and Be Miller JN, Academic Press, New York, 1962.
19. AOAC: Official Methods of Analysis. 15th ed, Arlington, 1990.


**How to cite this article**:
Fernandes B, Pereira L, Pereira T and Shruthi SD.: Evaluation of *Murraya* Extracts for Controlling Fungi Growth on *Zapota* through Estimation Studies. *Int J Pharm Sci Res*, 2012; Vol. 3(7): 2196-2200.