

Phytochemical Investigation Studies of Anti-Diabetic Phytochemicals of Plant *Murraya Koenigii* (Linn). Spreng belonging to Family Rutaceae

Ansari Yaasir Ahmed^{*1}, Mohd Farooque², Anwar Ahmad³, Md. Azhruddin⁴, M.S Sayyed⁵,

¹Lecturer at Jamia College of Pharmacy, Akkalkuwa, dist Nandurbar(M.S)-425415.

^{2,3,4,5}U.B.K.W.T's D.pharmacy college, Kunjkhedda dist Aurangabad(M.S).

Email: yasir.222@rediffmail.com¹

Abstract- The extract of (*Murraya koenigii* leaves) was subjected to preliminary phytochemical investigation and molecular docking studies. The molecular docking studies were carried out to find investigate the interaction of phytochemicals present in the leaf of the *Murraya koenigii*. The results of phytochemical investigation revealed the presence of alkaloids & the molecular docking studies showed good results. The preliminary phytochemical investigation has shown that alkaloids are the main constituents of this extract & the molecular docking studies have shown that most of the carbazole alkaloids have shown interaction especially Euchristine B has very good H-bond interaction with PDB ID:3W37 (-5.326), Koenimbine for 1IEI (-2.422) & 3W37 (-3.133).

Index Terms: extract, *Murraya koenigii*, phytochemicals, alkaloids.

1. INTRODUCTION

A natural product is a chemical compound produced by a living organism - found in nature that usually has a pharmacological or biological activity for use in pharmaceutical drug discovery and drug design. A natural product can be considered as such even if it can be prepared by total synthesis. These small molecules provide the source of inspiration for the majority of FDA-approved agents and continue to be one of the major sources of inspiration for drug discovery. In particular, these compounds are important in the treatment of life-threatening diseases. Natural products may be extracted from tissues of terrestrial plants, marine organisms or microorganism fermentation broths. A crude (untreated) extract from any one of these sources typically contains novel, structurally diverse chemical compounds, which the natural environment is a rich source of Chemical diversity in nature is based on biological and geographical diversity, so researchers travel around the world obtaining samples to analyze and evaluate in drug discovery screens or bioassays. This effort to search for natural products is known as bioprospecting. Plants have always been a rich source of lead compounds. Many of these lead compounds are useful drugs in themselves and others have been the basis for synthetic drugs. Clinically useful drugs which have been recently isolated from plants include the anticancer agent paclitaxel (Taxol) from the yew tree, and the antimalarial agent artemisinin from *Artemisia annua*.¹ Rutaceae, commonly known as the rue or citrus family, is a family of flowering plants, usually placed in the order Sapindales. Species of the family generally have flowers that divide into four or five parts, usually with strong scents. They range in form and size from herbs to shrubs and small trees. About 346 various plants belonging to family

Rutaceae have been identified & widely used as medicinal agents. The *Murraya* comprises 12 species in the family Rutaceae, including the Curry Tree. This genus, along with genera *Clausena* and *Glycosmis* within the same family are a major source of carbazole alkaloids. Parts of these trees are used in folk medicine and the leaves of *M.koenigii* are and ingredient in curry. The genus has important horticultural uses in landscaping, as well. Some of the important plants belonging to this Genus are *Murraya alata* Drake, *Murraya koenigii* (L.) Spreng (Curry Tree, Curry Patta), *Murraya ovatifoliolata* (Engl.) Domin, *Murraya paniculata* (L.) Jack (Orange Jessamine), *Murraya stenocarpa* (Drake) Swingle.

The curry leaf tree, *Murraya koenigii* (L) Spreng., is a member of the orange subfamily of the Rutaceae, and closely related to popular hedge plant, orange jasmine (*M. paniculata* Jack). The most common local name for the curry leaf tree in India is mitha nim, or mitha neem (meaning "sweet nim" or "sweet neem"), to distinguish it from the botanically unrelated but somewhat similar neem tree (*Azadirachta indica* A. Juss.). The latter has non-aromatic leaves eaten as a vegetable and is being widely exploited today for the insect-repellent and insecticidal action of the leaves and extracts of the seeds. There are numerous other dialectical names for the curry leaf in India including kathmin, kurry patta, kariaphulli, karayapan, barsanga, and glandla. The Ceylonese call it karivempu, karapinchu, or katu vempu. In Malaya, it is known as curry bush, garupillai, kerupulai, or karwa pale. In Vietnam it is xan troc, chum hoi trang, or sao nhon; in Laos, dok ki be, or dok khi be.³ The leaves are highly valued as seasoning in southern and west-coast Indian cooking, and Sri Lankan cooking, much like bay leaves, and especially in curries. They are also available dried, though the aroma is largely inferior. The leaves of *Murraya koenigii* are also used as a herb in Ayurvedic

medicine. Their properties include much value as an anti-diabetic, antioxidant, antimicrobial, anti-inflammatory, hepatoprotective, anti-hypercholesterolemic etc. Curry leaves are also known to be good for hair, for keeping it healthy and long. This plant is also reported to have stimulant, antidiarrhoeal, hypolipidaemic and antiatherosclerotic properties. Present investigation is planned to have a detailed study of the plant & search for still better phytochemicals present if any based upon the literatures available.

2. INTRODUCTION TO THE PLANT

Botanical Name-*Murraya koenigii* (L.) Spreng.

Family- Rutaceae

Vernacular names:

Karnataka- *karibevu*

English- Curry leaf-tree

Hindi- *kurry patta*

Morphology:

Flowers, bisexual, white, funnel-shaped, sweetly scented, stalked, complete, ebracteate, regular, actinomorphic, pentamerous, hypogynous, the average diameter of a fully opened flower being 1.12 cm; inflorescence, a terminal cyme, each bearing 60 to 90 flowers; calyx, 5-lobed, persistent, inferior, green; corolla, white, polypetalous, inferior, with 5 petals, lanceolate; length 5mm.

Pharmacological Properties-

Murraya koenigii has been mentioned in the traditional medicinal system Ayurveda (Sathyavati *et al.*, 1987). Bark, root, leaves, fruits and fruit pulp of *Murraya koenigii* are widely used in the treatment of diabetes, obesity, vomiting, constipation, indigestion, diarrhoea, dysentery, piles, nausea, to relieve kidney pain etc.

A few reports are available on the scientific probing to validate the pharmacological properties of *Murraya koenigii*. Some constituents of *Murraya koenigii* are reported to have anti fungal activity (Das *et al.*, 1965). Anti-spasmodic and anti-amoebic activity reported by Bhakuni *et al.* (1969) and Kong *et al.* (1986). Ramsewak, *et al.* (1999) and Rahman and Gray, 2005 reported antimicrobial activity. Antitrichomonal activity was reported by Adebajo *et al.* (2006.) The apoptotic activity of mahanini, pyrayafoline-D and murrifoline-I, corbozole alkaloids from *Murraya koenigii* in human myeloid cancer cell line HL-60 have been reported (Roy *et al.*, 2004; Ito *et al.*, 2006). The positive ionotropic effect of *Murraya koenigii* extracts reported by Narayana and Sastry, (1975) and Shah and Juvekar, (2006).

Antidiabetic Activity

Narayana and Sastry in 1975 reported the hypoglycemic activity of *Murraya koenigii*. The aqueous extract of leaves of *Murraya koenigii* after oral as well as intravenous administration to normal and alloxan diabetic dogs produced the hypoglycemia.

Santhakumari *et al.* in 1987 reported the hypoglycemic activity of crushed leaves of *Murraya koenigii* in rabbits, human volunteers and alloxan induced diabetic rats. Iyer and Mani in 1990 reported that curry leaves powder supplementation (12g providing 2.5 g fibre) to 30 non-insulin dependent diabetes mellitus patients for a period of 1 month resulted in the transient reduction in fasting and post-prandial blood sugar levels.

Phytochemistry Of *Murraya Koenigii*

The *Murraya koenigii* leaves having 66.3% moisture; 6.1 % protein; 1.0% fat (ether extract); 16.0% carbohydrate; 64.0 % fibre; 4.2% mineral matter; 810.0 mg calcium; 600.0 mg phosphorus; 3.1 mg Iron; 12600 i.u. carotene (as vitamin A); 2.3 mg nicotinic acid and 4 mg/100 g vitamin C. The leaves are devoid of thiamine and riboflavin. The leaves are a fair source of vitamin A, a rich source of calcium. Due to the presence of oxalic acid in high concentration (1.35% total oxalates; 1.15% soluble oxalates) the nutritional availability of calcium was affected. The free amino acids present in the *Murraya* leaves are asparagine, glycine, serine, aspartic acid, glutamic acid, 1955: Ananthasamy *et al.*, 1996; Wang, 2003; Maheswari and Subramanian, 2003; Math and Balasubramanian, 2005.

Table- 1 The methanol soluble phytochemical constituents of *Murraya koenigii* Spreng Leaf-

S. No	Chemical Constituents	Quantity (per 100gm)	Activity
1	Cadine ne (Sesquiterpene)	5.2%	Hypoglycemic (Tommasi et al., 1991), Hypotriglyceridemic (Rajendra and D'souza, 1998)
2	Carotene	12600i.u.	Hypocholesterolemic and antioxidant (Fuhrman et al., 2000)
3	cayophyllene	7.3%	Fungitoxic (Rajendra and D'souza, 1998)
4	Dipentene	15.9%	Hypoglycemic (Tommasi et al., 1991) and Fungitoxic (Rajendra and D'souza, 1998)
5	Element	7.09%	Fungitoxic (Rajendra and D'souza, 1998)
6	Fat	1%	Stored energy (Murray et al., 1996)
7	Gurjunene	NA	Fungitoxic (Rajendra and D'souza, 1998)
8	Mahanimbime	NA	Antioxidant (Scavenges superoxide radicals) (Ramsewak et al., 1999)
9	Mahanine	NA	Anti-inflammatory (Ramsewak et al., 1999)
10	Murrayanol	NA	Anti-inflammatory (Ramsewak et al., 1999)
11	Nicotinic Acid	2.3 mg	Enhances insulin secretion (Patole and Agte, 1998), decreases cholesterol, Triglyceride. LDL and VLDL (Satyanarayana, 1999)
12	Phellandrene	6.5%	Fungitoxic (Rajendra and D'souza, 1998)
13	Proline	NA	Glycogenic (West et al., 1967)
14	Resin	NA	Slower glucose adsorption and reduces LDL-C (Anderson et al., 1991)
15	Terpine	6.1%	Fungitoxic (Rajendra and D'souza, 1998)
16	Thujene		Fungitoxic (Rajendra and D'souza, 1998)

3. MATERIALS AND METHODS

Materials

Chemicals And Drugs Used During Study:

- Ascorbic Acid (ASC)
- Methanol (Nice – Cochin)
- Ethylene diamine tetra-acetic acid Disodium salt (EDTA) (SD Fine chemicals)

- Ferric chloride (SRL)
- Formalin (Nice – Cochin)
- Hydrochloric acid (SD Fine chemicals)
- N- butanol
- pH meter (Elico-India)
- Phosphate buffer
- Potassium chloride (KCl) (S.D. Fine Chemicals – Mumbai.)
- Potassium dichromate
- Trichloroacetic acid (SRL)

4. PLANT MATERIAL

The leaves of *Murraya koenigii* (L.) Spreng. was collected from around Dharwad district, Karnataka, India and was authenticated by Dr. S.S. Hebbar Lecturer in Biology, Govt. P.U. College, Dharwad (India). A voucher specimen has been kept in the herbarium of pharmacognosy.

5. METHOD:

Plant Material And Extraction:

The fresh leaves of *Murraya koenigii* were collected from the local market at Dharwad, Karnataka, India. The plant material was identified, authenticated and deposited in the herbarium at the Dept. of Botany, Karnataka University, Dharwad. The collected leaves were dried under shade and crushed to moderately coarse powder. The dried leaves (2 kg) were extracted with Hexane/ethyl acetate/95% methanol (MeOH) using Soxhlet apparatus. The MeOH extract was then concentrated under reduced pressure using rotavapour and acidified with 0.5 M H₂SO₄. The acidic extract was washed with chloroform to remove neutral components. The aqueous acidic fraction was then made basic with ammonia (pH 10) and extracted again with chloroform until the aqueous layer was free of alkaloids. The combined chloroform extracts were evaporated in rotary evaporator to yield total alkaloidal extract of *Murraya koenigii* leaves (MKA) as a dark brown residue (0.124% w/w of the dry starting material) (Rujjanawate et al., 2003). The presence of alkaloids were confirmed with preliminary phytochemical evaluation.

Preliminary Phytochemical Analysis:

➤ *Molisch's test:*

Treat the extract solution with few drops of alcoholic α -naphthol. Add 0.2 ml of Concentrated H₂SO₄ slowly through the sides of the test tube, **purple to violet** colored ring appears at the junction.

➤ *Benedict's test:*

Treat the extract solution with few drops of Benedict's reagent (alkaline solution containing cupric citrate complex) and upon boiling on water bath, **reddish brown** precipitate forms if reducing sugars are present.

General Test For Monosaccharides:

➤ *Barfoed's test:*

Heat the test tube containing 1ml reagent and 1 ml of extract solution in a beaker of boiling water; if **red cuprous oxide** is formed within two minutes, a monosaccharide is present. Disaccharides on prolonged heating (about 10 min) may also cause reduction, owing to partial hydrolysis to monosaccharide.

➤ *Selwinoff's test:*

Hydrochloric acid reacts with ketose sugar to form derivative of furfuraldehyde, which gives red colored compound when linked with resorcinol. Add extract solution to about 5 ml of reagent and boil. Fructose gives **red** color within half minute. The test is sensitive to 5.5 mmol/Lt. if glucose is absent. If glucose is present it is less sensitive and on addition of large amount of glucose it gives similar color.

➤ *Fehling's test:*

Equal volume of Fehling's A (Copper sulphate in distilled water) and Fehling's B (Potassium tartarate and Sodium hydroxide in distilled water) reagents are mixed along with few drops of extract solution, boiled, a **brick red** precipitate of cuprous oxide forms, if reducing sugars are present.

➤ *Caramelisation:*

Carbohydrates when treated with strong sulfuric acid, they undergo charring with the dehydration along with burning sugar smell.

➤ *Tollen's test:*

To 100 mg of extract add 2 ml of Tollen's reagent, a **silver mirror** is obtained inside the wall of the test tube, indicates the presence of aldose sugar.

6. RESULTS

In the present study the extract from leaves of *Murraya koenigii* (L.) Spreng. was subjected for phytochemical and pharmacological investigations. The present study revealed the following data.

Phytochemical Investigation

Preparation of fractions and properties:

The crude fraction of extract was obtained by rotary flash evaporation process with solvent chloroform.

Preliminary phytochemical screening:

As shown in table 5.1 Preliminary phytochemical investigation of extract showed presence of alkaloids.

Table- 2 Preliminary phytochemical analysis of EXTRACT:

Sr	TEST	CHLORO FORM EXTRAC T
1	Tests for Carbohydrates	-ve
2	Tests for Proteins & Amino acids	-ve
3	Tests for Sterols and Triterpenoids	-ve
4	Tests for Glycosides	-ve
5	Tests for Alkaloids	+ve
6	Tests for Phenolic Compounds	-ve
7	Tests for Flavonoids	-ve

Chromatographic Studies:

TLC of extract evaluation.

Stationary Phase: Silica gel G

Mobile Phase: Toluene: Chloroform: Ethanol

Proportion: 35.5:50:14.5

Detection: Dragandraff reagent.

Preparation of samples: extract was dissolved in chloroform.

Solvent front: 13.1 cm

No of spots: Three (0.3cm, 8.5 cm and 8.7 cm)

RF Values: 0.0229, 0.648 and 0.664

Fig. 1: TLC of extract



7. SUMMARY & CONCLUSION

The extract of (*Murraya koenigii* leaves) was subjected to preliminary phytochemical investigation. The results of phytochemical investigation revealed the presence of alkaloids & showed good results. The phytochemicals present as carbazole alkaloids in leaf were Mohanimbine,

Koenimbine, and Euchristine-B. All the 3 PDB IDs represent different classes of proteins which have direct effect on the Diabetes mellitus. DM is one of the most important health problems worldwide, showing high incidences of prevalence and mortality. DM can be defined as a group of metabolic disorder characterized by chronic hyperglycemia, resulting from defect in insulin secretion, insulin action or both giving rise to impaired function in the carbohydrate, lipid and protein metabolism.⁷⁸ The preliminary phytochemical investigation has show that alkaloids are the main constituents of this extract. Therefore these results open new platform for study of these compounds as antidiabetic agents.

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