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PHYTOCHEMICAL STUDIES AND QUALITATIVE ANALYSIS BY TLC OF MURRAYA KOENIGII BARK EXTRACT

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Abstract:

Murraya koenigii is a medium size, ever green plant which has been utilized as a source of food, medicine, and other agricultural purposes in different communities. Thus, the preliminary phytochemical analysis and TLC separation was done using methanol, n-hexane, and ethyl acetate(1:3:1), as solvent system while iodine vapour as spotting agent. The phytochemical screening of diethyl ether extracts of bark revealed the presence of carbohydrates , anthraquinones glycosides ,saponins ,flavanoids, and alkaloids, while chloroform extracts of bark revealed carbohydrates, tannins, saponins, and alkaloids, while acetone extracts of bark revealed the presence of carbohydrates, anthraquinones glycosides, flavanoids and alkaloids,while ethanol extracts of bark revealed the presence of carbohydrates, tannins, anthraquinones glycosides,s aponins, flavanoids and alkaloids.TLC separation showed (3) spots each of Diethyl Ether, Chloroform, Acetone, Ethanol from bark extracts. From our findings, it can be concluded that Murraya Koenigii contains some significant phytochemicals that can exhibit desired therapeutic activities such as Antioxidant, Anti-Microbial, Anti-Fungal, Anti-Diabetic, Anti-Ulcer and Cosmetic use. However, there is a need to conduct further Pharmaceutical Analysis on test extracts in order to establish these biomedical applications.

Keywords: *Thin Layer Chromatography, Murraya koenigii Bark, Phytochemical screening.*

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INTRODUCTION:

Murraya koenigii, commonly known as *curry* leaf or *kari patta* in Indian dialects, belonging to Family Rutaceae which represent more than 150 genera and 1600 [1]. Murraya Koenigii is a highly values plant for its characteristic aroma and medicinal value. It is an important export commondity from India as it fetches good foreign revenue. A number of chemical constituents from every part of the plant have been extracted. The most important chemical constitutents responsible for its intense characteristic aroma are P-gurjunene, P-caryophyllene, P-elemene and O-phellandrene. The plant is rich source for carbazole alkaloids [2]. Bioactive coumarins, acridine alkaloids and carbazole alkaloids from family Rutaceae were reviewed by Ito3. *M. koenigii* is widely used in Indian cookery for centuries and have a versatile role to play in traditional medicine.

The plant is credited with tonic and stomachic properties. Bark and roots are used as stimulant and externally to cure eruptions and bites of poisonous animals. Green bark are eaten raw for cure of dysentery, diarrhoea and for checking vomiting. Bark and roots are also used traditionally as bitter, anthelmintic, analgesic, curing piles, inflammation, itching and are useful in leucoderma and blood disorders,. Several systematic scientific studies are also being conducted regarding the efficacy of whole plant or its parts in different extract forms for the treatment of different diseases. *M. koenigii* contains a number of chemical constituents that interact in a complex way to elicit their pharmacodynamic response. A number of active constituents responsible for the medicinal properties have been isolated and characterized.

This plant has been reported to have anti-oxidative, cytotoxic, antimicrobial, antibacterial, anti ulcer, positive inotropic and cholesterol reducing activities. Therefore the present review summarizes the available literature till date on isolation of phytoconstituents, biological activities of the isolated compounds and pharmacological actions of extracts along with the clinical studies.Fresh bark, dried leaf powder, and essential oil are widely used for flavouring soups, curries, fish and meat dishes, eggs dishes, traditional curry powder blends, seasoning and ready to use other food preparations [3]. Koenoline (1- methoxy-3- hydroxy methyl carbazole) was isolated form the root bark65, Mukoline, mukolidine were isolated form the benzene extract of roots [4].Stem showed the presence of mukeic acid (1- methoxy carbazole- 3-carboxylic acid)77 and mukoeic acid [5]. The fresh bark of Murraya koenigii from Dehradun89 contains apinene (51.7%), sabinene (10.5%), ß-pinene (9.8%), ß- caryophyllene (5.5%), limonene (5.4%), bornyl acetate (1.8%), terpinen-4-ol (1.3%), g-terpinene (1.2%) and a-humulene (1.2%) as the major constituents [6]. Aqueous and methanolic extracts of bark and fruits of *M. koenigii* showed very good antidiabetic activity in alloxan-induced diabetic rats. Plasma insulin was observed with significantly high levels on the 43rd and 58th days of treatment in aqueous and methanol extracts of *M. koenigii* -treated groups [7]. Curry leaf oil cream showed the low sun protection factor (2.04±0.02), so the cream can be used in maintaining the natural skin pigmentation or it can be used as additives in other formulations to enhance the activity [8]. Mahanimbine a chemical constituent of M. koenigii was isolated from column chromatography of the petroleum ether extract of dried plant [9]. Mahanimbine, murrayanol and mahanine from fresh bark showed anti microbial and topoisomerase I and II inhibitory activity. Marmesin- 1'-O-*β*-D-galactopyranoside from stem bark showed anti bacterial, anti viral and anti fungal activity[10]. Antiulcer activity of aqueous and ether extracts of M. koenigii was studied in reserpine induced gastric ulcer model in albino rats. Extracts were effective in gastric ulceration and suggested as protective as ranitidine [11].

MATERIALS AND METHODS:

These include the test plant (the fresh bark *Murrayakoenigii*), beaker, conical flask, measuring cylinder (large and small), glass funnel, glass stirrer, cotton wool, spatula, bunsen burner, top mettler weighing balance, test tubes, stainless steel tray, thermostat water bath, oven, syringe and needle, aluminum foil paper, hand gloves, mortar and pestle, analytical weighing balance, test-tube holder, refrigerator, meter rule, sieves (No. 5), bottles, UV fluorescence analysis cabinet tripod stand, wire gauze, capillary tubes, retort stand, thin layer chromatography (TLC) paper, TLC tank, test tube rack, tiles and filter paper.

Collection of Plant

Fresh and healthy *Murrayakoenigii* (Curry bark) were collected from the local market. It was ensured that they were healthy, uninfected and they were thoroughly washed and rinsed with sterile distilled water.

Authenticationand Processing Of Curry Bark:

The curry bark of murraya koenigii is collected and identified and authesicated by taxonomist. The plant materials were dried under shade at our Pharmaceutical analysis Laboratory for about four weeks and then made into powdered form, using mortar and pestle and then sieved.

Extraction

250 grams of powdered bark of *Murrayakoenigii* were extracted using 250ml of different solvents like Diethylether,Chloroform,Ethanol,Acetone in Soxhlet apparatus separately for 24 hours and they were concentrated by evaporation process. The cold extracts thus obtained were filtered with Whatman No. 1 filter paper into different conical flask and allowed to dry at room temperature under normal

atmospheric pressure. The obtained crude extracts were stored in closed container and used for preliminary qualitative phytochemical analysis. Bark extraction process shown in Tables 1 and 2.

Extractive value = weight of plant (part) extract/weight of dry powdered Sample × 100
Volume of ethanol used = 1L
Weight of dried powdered = 250g
Weight of ethanol extract = 101g
Extractive value = 40.4%

Table 1: Results of Bark Extraction

Parameters	Diethyl ether	Chloroform	Acetone	Ethanol
Volume of solvent used (ml)	250	250	250	250
Weight of dried powdered (g)	200	200	200	200
Weight of solvent extracts (g)	5.5	4.3	5	4.2
Extractive value (%)	2.68	2.15	2	2.10

Table 2: Partition of Bark Extract

Parameters	Diethyl ether	Chloroform	Acetone	Ethanol
Volume of solvent used for	150	150	150	150
Weight of partitioned solvent	47.6	18.8	9.9	20.8

Phytochemical Analysis
Phytochemical analysis for the qualitative detection of alkaloids, anthraquinone, carbohydrates, flavonoids, tannins and saponins was carried out on the extracts as described by Trease and Evans (2010), Sofowora (1993) and Harbone (1973).Various phytochemical tests such as alkaloids, carbohydrates, glycosides, saponins, phytosterols, phenols, tannins, flavanoids, diterpenes, proteins and amino acids were performed using bark extract of *Murrayakoenigii*. The extract was subjected to determination of preliminary qualitative phytochemical screening.

Thin layer chromatography (TLC)
Commercially available standard TLC plate was used with standard particle size range to improve reproducibility. The absorbent silica gel coated on an TLC plate of 7.3 cm length, 2.5 cm breadth and 0.3 cm thick plate. Small spot of the solution containing the sample was applied on the plate 1.0 cm from the bottom marked.

Spotting and development, Visualization and Detection
The sample spotted on the plate was allowed to dry before the plate was placed into the chromatographic tank which is completely saturated with mobile phase. The reaction was then monitored as the solvent moved up the plate (elutes the sample) using mobile phase solvent ratio 1:3:1 of methanol, n-hexane and ethyl acetate, respectively. When the solvent reaches the top of the plate, it is removed, marked and dried. Following separation of the solvent, the plate was removed and dried; the spots detected using various techniques and reagents. This includes visualization in daylight; viewing under UV at 254 and 366 nm i.e. short and long wavelengths and spraying with spotting reagent, using iodine vapor tank. The phytochemical screening of diethyl ether extracts of bark revealed the presence of carbohydrates , anthraquinones glycosides ,saponins ,flavanoids, and alkaloids, while chloroform extracts of bark revealed carbohydrates, tannins, saponins, and alkaloids, while acetone extracts of bark revealed the presence of carbohydrates, anthraquinones glycosides, flavanoids and alkaloids, while ethanol extracts of bark revealed the presence of carbohydrates, tannins, anthraquinones glycosides, saponins, flavanoids and alkaloids.

RESULT AND DISCUSSION:

The results of the qualitative phytochemical analysis of *Murrayakoenigii* bark extract are depicted in Table .3 to Table .8. The preliminary phytochemical screening of Acetone extract of *Murrayakoenigii* bark revealed the presence of multiple chemical constituents. The phytochemical screening of diethyl ether extracts of bark revealed the presence of

carbohydrates , anthraquinones glycosides ,saponins ,flavanoids, and alkaloids, while chloroform extracts of bark revealed carbohydrates, tannins, saponins, and alkaloids, while acetone extracts of bark revealed the presence of carbohydrates, anthraquinones glycosides, flavanoids and alkaloids, while ethanol extracts of bark revealed the presence of carbohydrates, tannins, anthraquinones glycosides, Saponin, flavanoids and alkaloids. The compounds of bark extract using different solvent were separated using TLC and have to test for therapeutic purposes that shown inTables 14 to 16 shows TLC results of bark extract.

Table 3: Test for carbohydrate:

Test	Extracts			
	Diethyl ether	Chloroform	Acetone	Ethanol
Molisches test (for carbohydrates)	-	+	+	+
Iodine test (for starch)	+	+	+	+
Fehling's test (for reducing sugar)	+	+	+	+
Combined reducing sugar test	+	+	-	+
Barfoed test (for monosaccharides)	-	+	+	-

Table 4: Tests for tannins (hydrolysable and condensed):

Test	Extracts			
	Diethyl ether	Chloroform	Acetone	Ethanol
Lead sub- acetate test	+	-	-	+
Ferric chloride test	-	+	+	+
Bromine water test	-	+	-	-

Table 5:Tests for anthraquinones glycosides:

Test	Extracts			
	Diethyl ether	Chloroform	Acetone	Ethanol
Borntrager's Test	+	-	+	+

+ Abundance, - Absence

Table 6:Tests for Saponin:

Test	Extracts			
	Diethyl ether	Chloroform	Acetone	Ethanol
Frothing test	+	+	-	+
Haemolysis test	+	+	+	+

+ Abundance, - Absence

Table 7:Tests for Flavanoids:

Test	Extracts			
	Diethyl ether	Chloroform	Acetone	Ethanol
Shinoda's test	-	-	-	-
Ferric chloride test	+	+	+	+
Lead acetate test	-	-	+	-
Sodium hydroxide test	+	-	-	+

+ Abundance, - Absence

Table 8: Tests for alkaloids:

Test	Extracts			
	Diethyl ether	**Chloroform**	**Acetone**	**Ethanol**
Mayers reagent	-	+	+	+
Dragendorff A × B	+	+	+	+
Wagners reagent	+	+	+	+
10% w/v tannic acid	+	+	-	+
1% w/v picric acid	-	+	+	-

+ Abundance, - Absence

Table 9: Diethyl ether extracts TLC results:

Spots position (cm)	Rf values (cm)	Day light	UV-254nm	UV-366nm	Iodine vapour
2.5	0.4	white	blue	violet	light purple
2.3	0.3	cream	light blue	light violet	purple
3.5	0.66	cream	light blue	light violet	purple

Table 10.Chloroform extract TLC results:

Spots position (cm)	Rf values (cm)	Day light	UV-254nm	UV-366nm	Iodine vapour
3.4	0.6	brown	white	brown	brown
2.5	0.44	light brown	violet	brown	yellow
3.5	0.66	light brown	white	brown	yellow

Table 11.Acetone extract TLC results:

Spots position (cm)	Rf values (cm)	Day light	UV-254nm	UV-366nm	Iodine vapour
3.5	0.77	green	green	blue black	yellow
4	1.77	dark green	dark brown	blue black	yellow
3	0.56	light green	light brown	blue black	yellow

CONCLUSION:

The phytochemical screening of diethyl ether extracts of bark revealed the presence of carbohydrates , anthraquinones glycosides ,saponins ,flavanoids, and alkaloids, while chloroform extracts of bark revealed carbohydrates, tannins, saponins, and alkaloids, while acetone extracts of bark revealed the presence of carbohydrates, anthraquinones glycosides, flavanoids and alkaloids,while ethanol extracts of bark revealed the presence of carbohydrates, tannins, anthraquinones glycosides,s aponins, flavanoids and alkaloids. Keeping in view the tremendous pharmacological activities and wealth of literature available, *M. koenigii* may be utilized to alleviate the symptoms of variety of diseases as evident form the pre-clinical data. Although crude extract from various parts of curry neem have numerous medical applications, modern drugs can be developed after extensive investigation of its bioactivity, mechanism of action, pharmaco-therapeutics, toxicity and after proper standardization and clinical trials. The available literature and wide spread availability of *M.koenigii* in India thus makes it an attractive candidate for further pre-clinical and clinical research.

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CONFLICT OF INTEREST STATEMENT

There is no conflict of interest associated with the authors of this paper.

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