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Phytochemical Screening and Thin Layer Chromatographic analysis for Antioxidant activity of *Murraya koenigii* (Curry leaf)

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Abstract

Plants have been one of the most important sources for producing medicines since ancient times. Hence, this study was focused on phytochemical screening of *Murrayakoenigii*and determination of antioxidant activity using Thin Layer Chromatographytechnique. The leaves of *Murrayakoenigii* were collected and subjected for crude extraction using Acetone solvent. The crude extract were analysed for the presence of phytochemicals. The results of phytochemical analysis of *Murrayakoenigii* leaf extractshowed the presence of Carbohydrates, Glycosides, Saponins, Phytosterols, Flavonoids and Diterpenes. R_f values of leaf extract of *Murrayakoenigii*(Acetone solvent) were calculated using TLC technique. TLC has been carried out on leaf extract of *Murrayakoenigii*(Acetone solvent) which had shown different $R_f$values. The extract which showed a promising result were subjected for column fractionation and the collected fractions were used for spot assay to determine antioxidant activity. The results of the antioxidant activity of *Murrayakoenigii*leaf extract revealed that plant possess antioxidant activity.

Key words: *Murraya koenigii* leaves, Phytochemical screening, Antioxidant, Spot assay and TLC.

Introduction

Plants since the ancient times have played a most important role in development of modern medicine. It has evolved through various forms like the complementary methods of Siddha, Ayurveda, Unani etc. to cure variety of diseases (Golla*et al.*, 2011 and Rawat, 2003). Nature endures its ways in supporting human activities in producing these medicinal compounds. They produce compounds called as secondarymetabolites that emulate a vital role in drug discovery. Plants derived drugs have found extensively used in most countries because they are easily accessible, safer and cheaper. At present, there are more number of lifesaving drugs derived from plants. More than 2000medicinal plant species are used in different forms to treat diseases. Thus medicinal compounds of plants were found as a curative for most disorders, apart from which they are well known for their tremendous effects of biological activities like antibacterial, antifungal, anticancer etc. which escalate the interest of human beings to ameliorate their studies in this field. Safe, non-toxic and dependable nature has drawn the attention of researchers.

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The screening of plant extracts has been of great interest to scientists in the search for new drugs for greater effective treatment of several diseases (Dinmayuga and Garcia, 1991). Therefore, plant extracts and phytochemicals with known antimicrobial properties can be of great significance in therapeutic treatments (Diallo *et al.*, 1999 and Rojas *et al.*, 2006). The most important bioactive compounds of plants are alkaloids, flavonoids, tannins and phenolic compounds (Edeoga*et al.*, 2005). Many plant leaves have antimicrobial principles such as tannins, essential oils and other aromatic compounds (Kumar and Singh, 1984). Some of the phytochemicals can significantly reduce the risk of cancer due to polyphenol antioxidant and anti-inflammatory effects. Some preclinical studies suggest that phytochemicals can prevent colorectal cancer and other cancer (Michaud *et al.*, 2000 and Greenberg *et al.*, 1994).

Antioxidants act against oxidative damage caused by Reactive Oxygen Species (ROS) and implies the importance of antioxidants and necessity for the discovery of new antioxidant compound from various sources. Several screening assays are available for the screening of potential antioxidants, Thin Layer Chromatography (TLC) bioautography assay is the best

method among them (Moore *et al.,* 2006). Comparing with other available methods, TLC bioautography is quick, convenience, simple and efficient method as active components form a complicated plant extract is also possible. So as, for the screening of antioxidants, the TLC bioautography assay is the good choice (Olech*et al.,* 2012 and Cimpoiu,2006). Several TLC techniques are developed and used for qualitative and quantitative analysis of antioxidants (Zhao *et al.,* 2010) but these methods use stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) as deviating agent (Kusznierewicz*et al.,* 2012).

Murrayakoenigii, commonly known as curry leaves, is a small spreading shrub, locally known as Karivepu. The species is a native of India (Bhattacharjee*et al.,* 2011) found throughout India, Srilanka, Bangladesh and Andaman Islands. The leaves are particularly associated with South Indian cuisines. The bark and the roots of curry leaves are used as a stimulant by the physicians. They are also used externally to cure eruptions and the bites of poisonous animals (Mhaskar*et al.,* 2000). It is also useful in Leucoderma and blood disorders (Kesari*et al.,* 2005) and the infusion of the washed leaves stops vomiting. Its nutritional value benefits both their young and the old alike. Women who suffers from calcium deficiency, osteoporosis etc. can find an ideal natural calcium supplement in curry leaves. Fresh juices of curry leaves, with lime juice and sugar, is an effective medicine in the treatment of morning sickness, nausea and vomiting due to indigestion and excessive use of 53 fats (SumanSing *et al.,* 2014).

Thus, main objective of the present study is to analyze phytochemical screening of *Murrayakoenigii*(Curry leaves) and antioxidant assay using TLC technique.

Material and Methods

Collection of plant

Fresh and healthy *Murrayakoenigii*(Curry leaves) 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.

Authentication of plant

The fresh leaves of *Murrayakoenigii*were authenticated by the taxonomist Prof. Jayaraman (Certificate No. PARC/2016/3222).

Extraction of plant

50 grams of powdered leaves of *Murrayakoenigii* were extracted using 250ml of Acetone solvent in Soxhlet apparatus separately for 24 hours and they were concentrated by evaporation process (Harborne, 1998 and Biren Shah, 2010). The obtained crude extracts were stored in closed container and used for preliminary qualitative phytochemical analysis.

Screening of phytochemicals

The phytochemical tests were carried out in the extract using the standard procedures as described by Sofowara (1993), Trease and Evans (1989) and Harbone (1973).

Various phytochemical tests such as alkaloids, carbohydrates, glycosides, saponins, phytosterols, phenols, tannins, flavanoids, diterpenes, proteins and amino acidswere performed using leaf extract of *Murrayakoenigii*. The extract was subjected to preliminary qualitative phytochemical screening for the determination of various primary and secondary metabolites(Table:1).

Thin Layer Chromatography (TLC) analysis of *murraya koenigii*leaf extract

The Thin layer chromatography were performed using the acetone solvent extract on analytical plates over Silica Gel-G of 0.2 mm thickness. These plates were developed mobile phase using Benzene:Ethanol(9:1) (Jaiswal*et al.,* 2011). The spot were visualized by exposing the plates to iodine vapor and R_f values were calculated.

TLC technique for antioxidant activity

The extract were subjected to TLC plate with the combination of Benzene:Ethanol (9:1). The developed plates were dried and sprayed with 0.05% of 2,2-diphenyl-1-picrylhydrazyl (DPPH) to identify the antioxidant compounds. The R_f value of the samples were calculated (Wang *et al.,* 2012).

Results and Discussion

The results of the qualitativephytochemical analysis of *Murrayakoenigii* leaf extract are depicted in Table: 2.The preliminary phytochemical screening of Acetone extract of *Murrayakoenigii*leaves revealed the presence of multiple chemical constituents. The results of the study showed the presence of Carbohydrate which was confirmed by Molisch's test by the appearance of Violet colour ring, Benedict's test by the appearance of Orange precipitate and Fehling's test by the appearance of Red precipitate. The presence of Glycosides was confirmed by Modified Borntrange's test by the appearance of Red brown colour and Legal's test by the appearance of Red colour. The presence of Saponins was confirmed by Foam test by the appearance of Foam formation. The presence of Phytosterols was confirmed by Salkowshi's test by the formation of Golden yellow colour. The presence of Flavonoids was confirmed by Alkaline reagent test by the appearance of Yellow colour and Lead Acetate test by the formation of Yellow colour. The presence of Diterpenes was confirmed by Copper acetate test by

the appearance of Green colour.The results of the study is in accordance with the reports of Bonde*et al.* (2011), who highlighted various ethnobotanical and traditional use as well as phytochemical and pharmacological reports of *Murrayakoenigii.*

DPPH method measures electron-donating activity (free radical scavenging activity) of compounds and provides an evaluation of antioxidant activity (Prema*et al.,* 2012). Antioxidant potential compounds of *Murraya koenigii* leaf extract using TLC plates were identified insitu with the DPPH reagent. The formation of yellowish bands on the purple background were considered as antioxidants. The antioxidant potential of combined plant extracts of *Cissus quadrangularis* &*Aegle marmelos*was reported by TLC autobiography (Ajayi*et al.,* 2011). Thus, acetone extract of *Murraya koenigii*s howed formation of distinct bands at the regions of R_f values 0.25, 0.35 and 0.51 (Table: 3). There is a growing interest in the investigation of natural antioxidant compounds from plants, since they contain secondary metabolites with structural diversity (Joseph and Priya, 2011).

Conclusion

Thus to conclude the leaf extract of *Murrayakoenigii*using acetone revealed the presence of various phytochemical compounds such as carbohydrates, glycosides, saponins, phytosterols, flavonoids and diterpenes as evidenced in the present study and also antioxidant properties. The compounds of leaf extract using acetone solvent were separated using TLC and were tested for their antioxidant properties by exposing it to DPPH assay. This study demands further research on these compounds for various therapeutic purposes.

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Table 1: Procedure of Phytochemical screening of *Murraya koenigii* Leaf extract

TESTS	RESULTS

For Alkaloids 1. Mayer's Test 1 ml of extract + 2-3 drops of Mayer's reagent 2. Drangendroff's Test 1 ml of extract + 2-3 drops of Drangendroff's reagent 3. Hager's Test 1 ml of Extract + 2-3 drops of Hager'sreagent	Yellow coloured precipitate Red precipitate Yellow coloured precipitate
For Carbohydrates 1. Molisch's Test 2 ml of extract + 2-3 drops of Molisch's reagent + Con. Sulphuric acid 2. Benedict's Test Extract + Benedict's reagent + heat 3. Fehling's Test 1 ml of extract + Fehling's A + Fehling's B + heat	Violet coloured ring at the juncture Orange red precipitate Red precipitate
For Glycosides 1. Modified Borntrager's Test 1 ml of extract + 2 ml of Ferric Chloride + Boil + Benzene + Ammonia 2. Legal's Test 1 ml of extract + Sodium nitroprusside + Pyridine + Sodium hydroxide	Rose-Pink colour in the ammonical layer Pink to blood red colour
For Saponins 1. Foam Test 1 ml of extract + Distilled water + Shake continuously for 5 minutes	Foam formation
For Phytosterols 1. Salkowski's Test 1 ml of extract + chloroform + Conc. Sulphuric acid + Shake	Golden yellow colour
For Phenols 1. Ferric Chloride Test Extract + 3-4 drops Ferric Chloride	Bluish black colour
For Tannins 1. 2 ml of extract + Ferric chloride	Blue colour – hydrolysable tannins Green colour – Condensed tannins
For Flavonoids 1. Alkaline Reagent Test 2 ml of extract + 2 ml of Sodium Hydroxide 2. Lead Acetate Test 2 ml of extract + 2 ml of Lead Acetate	Yellow colour Yellow colour
For Proteins & Amino Acids 1. Xanthoproteic Test 1 ml of extract + Conc. Nitric Acid 2. Ninhydrin Test 1 ml of extract + 0.25% Ninhydrin reagent + Heat	Yellow colour Blue Colour
For Diterpenes 1. Copper Acetate Test 2 ml of extract + 3-4 drops Copper Acetate	Green colour

Table 2: Qualitative Phytochemical Screening of *Murraya koenigii* (Curry leaf)

S.NO	CHEMICAL COMPOUNDS	ACETONE SOLVENT
1.	**Detection of Alkaloids**	
	Mayer's Test	-
	Dragendroff's Test	-
	Hager's Test	-
2.	**Detection of Carbohydrates**	
	Molisch's Test	+
	Benedict's Test	+
	Fehling's Test	+
3.	**Detection of Glycosides**	
	Modified Borntranger's Test	+
	Legal's Test	+
4.	**Detection of Saponins**	
	Foam Test	+
5.	**Detection of Phytosterols**	
	Salkowshis Test	+
6.	**Detection of Phenols**	
	Ferric Chloride Test	-
7.	**Detection of Tannins**	
	Ferric Chloride Test	-
8.	**Detection of Flavanoids**	
	Alkaline Reagent Test	+
	Lead Acetate Test	+
9.	**Detection of Proteins &Aminoacids**	
	Xanthoproteic Test	-
	Ninhydrin Test	-
10.	**Detection of Diterpenes**	
	Copper Acetate Test	+

Table 3: R_f value

TLC BAND	R_fValue
3rd Band	0.25
4th Band	0.35
5th Band	0.51