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Invitro Analysis of Phytochemicals of *Murraya Koenigii*

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Abstract

Murraya Koenigii which belong to the Rutaceae family has great antioxidant activity which is commonaly called as"Curry Patta", Metha Neem" consisting of 150 genera and 1600 species (satyavati et al, 1987). This study show that the *Murraya Koenigii* has the richest source of antioxidant such as Alkaloides, Flavonoids, Tannins, Glycosides, Saponins, Terpenoids, Protein, Carbohydrate, Phenol, and Coumarin. But Amino acid wad not found in the Murraya Koenigii. *Murraya Koenigii* plant has used as medicinal plant and food flavoring and spicing condiment.

Keywords: Murraya Koenigii, Alkaloids, Flavonoids

Abbreviation: MKL, MKF, MKS, MKR (Murraya Koenigii, Leaf, Fruit, Stem, Root respectively), MK1, MK2, MK3, MK4 (*Murraya Koenigii* Assam, Hisar, Hyderabad and Rudraprayag respectively), + stand for Present and – stand for Absent respectively.

1. Introduction

Murraya Koenigii which belong to the Rutaceae family has great antioxidant activity which is commonaly called as"Curry Patta", Metha Neem" consisting of 150 genera and1600 species (satyavati et al, 1987). It commonly is observed in foothills of Himalaya, Assam, Sikkim, Kerala, Tamin Naidu, Andhra Pradesh and Maharashtra (Bhattacharjee, 2001). It has the highest concentration of carbazole alkaloids. Carbazole alkaloids have also been shown to have anticonvulsant, anticancer, anti-inflammatory, diuretic,antiviral and other pharmacological properties (Knolker et al, 2008). Mahanine, Koenine, Koenigine and Koendine are alkaloids found in the leaves. Girinimbine, Mahanimbine and Murrayanine are the stem components. Curry plant roots have been found to contain carbazole alkaloids includes as Mahanimboline, Girinimbine. The fruits carbazole alkaloids are consisting Pyrayaquinone-A, Koenimbine, Murrafoline, Murrayazoline (Iyer *et al*, 2008)

2. Material & Method

Fresh leaf, stem, root and fruit of *Murraya Koenigii* have been collected from different eco-geographical position in Hisar, Assam, Rudraprayag and Hyderabad region in month of May-June fruiting season.

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Figure 2.1

Figure 2.2

Figure 2.3

Figure 2.4

Figure 2.1 *Murraya Koenigii* Plant Hyderabad,Telengana Region, Figure 2.2 *Murraya Koenigii* Plant Kamrup, Assam Region, Figure 2.3 *Murraya Koenigii* Plant Hisar, Haryana, Region, Figure 2.4 *Murraya Koenigii* Plant Rudraprayag Uttraakhand, Region

Extract process of *Murraya Koenigii*(Leaf, stems, roots & fruits)

The fresh sample was cut from the *Murraya Koenigii* plant, washed thoroughly three times in water, shade dried at room temperature. After complete drying, the leaves, stem, root and fruits were subjected to size reduction in a grinder and then sieved through sieve to get a coarse powder and stored in airtight glass jar. Coarse powder (25g) was extracted with solvent (500 ml methanol & 500 ml acetone) for 6 hours using reflux apparatus of extraction at boiling temperature. After the completion of extraction process, the solvents were evaporated, leaving a small yield of extracted plant material. The extract was further dried at room temperature until the remaining solvents were completely evaporated. The extract was stored at 4°C until further tests were carried out.

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Preliminary Phytochemical Tests:

Qualitative chemical tests of 10 mg extract were subjected to various chemical tests to detect various phytoconstituents.

Test for Alkaloids:

Dragendorff's test: To the extract add Dragendorff's reagent, reddish brown precipitate indicates presence of alkaloids.

Mayer's test: To the extract add Mayer'sreagent, cream colored precipitate indicates presence of alkaloids.

Wagner's test: To the extract add Wagner'sreagent, reddish brown precipitate indicates presence of alkaloids.

Hager's test: To the extract add Hager'sreagent, yellow precipitate indicates presence of alkaloids.

Test for Aminoacids:

Ninhydrine test: To the extract add Ninhydrine solution, boil, violet colour indicates presence of amino acid.

Test for Flavonoids:

Lead acetate test: To 1 ml of extract, 1 to 2 drops of lead acetate solution was added. Formation of yellow colored precipitated indicated the presence of flavanoid.

Alkaline reagent test: To the extract add few drops of sodium hydroxide solution, intense yellow colour is formed which turns to colorless on addition of few drops of dilute acid indicate presence of flavonoids layer turns green which shows presence of steroids and formation of deep red colour indicates presence of triterpenoids.

Salkowski test: Treat the extract with few drops of concentrated sulphuric acid red colour at lower layer indicates presence of steroids and formation of yellow coloured lower layer indicates presence of triterpenoids.

Test for Carbohydrates:

Molish's test: To the extract add few drops of alcoholic α-naphthol, then add few drops of concentrated sulphuric acid through sides of test tube, purple to violet colourring appears at the junction.

Test for Coumarin glycosides:

Place a small amount of sample in test tube and cover the test tube with a filter paper moistened with dilute sodium hydroxide solution. Place the covered test tube on water bath for several minutes. Remove the paper and expose it to ultraviolet (UV) light, the paper shows green fluorescence.

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Test for Steroids and Triterpenoids:

Libermann-Burchard test: Treat the extract with few drops of acetic anhydride, boil and cool. Then add concentrated sulphuric acid from the side of the test tube, brown ring is formed at the junction two layers and upper layer turns green which shows presence of steroids and formation of deep red colour indicates presence of triterpenoids.

Salkowski test: -Treat the extract with few drops of concentrated sulphuric acid red colour at lower layer indicates presence of steroids and formation of yellow coloured lower layer indicates presence of triterpenoids.

Test for Carbohydrates:

Molish's test: To the extract add few drops of alcoholic α-naphthol, then add few drops of concentrated sulphuric acid through sides of test tube, purple to violet colourring appears at the junction.

Test for Coumarin glycosides:

Place a small amount of sample in test tube and cover the test tube with a filter paper moistened with dilute sodium hydroxide solution. Place the covered test tube on water bath for several minutes. Remove the paper and expose it to ultraviolet (UV) light, the paper shows green fluorescence.

Test for Saponin glycosides:

Froth formation test: To 1 ml of the extract,1-2 drops of distilled water was added and shaken vigorously until persistent form was observed.

Test for Saponin glycosides:

Foam test: To 1 ml of the extract, 1-2 drops or distilled water was added and shaken vigorously until persistent foam was observed.

3. Result & Discussion

Results of Phytochemical screening:

The results of phytochemical screening of methanolic extracts and acetone extract of fruit, stem, leaf, and root samples of *Murraya Koenigii* which were collected form Hyderabad, Hisar, Assam, and Rudraprayag shows in table 1-4.

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Table 1: Preliminary phytochemical tests of MK3 Plant

S. No	Phyto-constituents	Chemical Tests	MKF		MKL		MKS		MKR	
			Acetone	Methanol	Acetone	Methanol	Acetone	Methanol	Acetone	Methanol
1	Alkaloides	Wagner's Test	-ve	+ve	-ve	-ve	-ve	+ve	-ve	+ve
		Dragendroff's Test	+ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve
		Mayer's Test	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
		Hager's Test	-ve	-ve	+ve	-ve	-ve	-ve	-ve	-ve
2	Flavonoids	Alkaline Test	+ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve
		Lead Acetate Test	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
3	Tannins	Ferric Chloride	-ve	+ve	+ve	+ve	-ve	+ve	-ve	+ve
4	Glycosides	$FeCl_3$	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
5	Saponins	Foam	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
6	Terpenoids	Libermann-Burchard Test	+ve	+ve	-ve	-ve	+ve	+ve	+ve	+ve
		Salkowski Test	+ve	-ve	-ve	-ve	+ve	-ve	+ve	-ve
7	Protein	Warming Test	+ve	-ve	+ve	+ve	+ve	-ve	+ve	-ve
		Biuret Test	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
8	Carbohydrate	Molish Reagent Test	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
9	Phenol	Ferric Chloride	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
1	Amino	Ninhydrine	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve

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0	Acid									
11	Coumarin	Coumarin	+ve	+ve	-ve	-ve	+ve	+ve	+ve	+ve

Table 2: Preliminary phytochemical Tests of MK2 Plant

S. No	Phyto constituents	Chemical Tests	MKF		MKL		MKR		MKS	
			Acetone	Methanol	Acetone	Methanol	Acetone	Methanol	Acetone	Methanol
1	Alkaloides	Wagner's Test	+ve	-ve	+ve	-ve	-ve	-ve	+ve	+ve
		Dragendroff's Test	+ve	-ve	+ve	+ve	+ve	-ve	+ve	-ve
		Mayer's Test	-ve	-ve	-ve	-ve	-ve	+ve	-ve	-ve
		Hager's Test	+ve	+ve	+ve	-ve	-ve	-ve	-ve	-ve
2	Flavonoids	Alkaline Test	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
		Lead Acetate Test	+ve	+ve	-ve	+ve	-ve	-ve	+ve	+ve
3	Tannins	Ferric Chloride	-ve	+ve	-ve	+ve	-ve	+ve	-ve	-ve
4	Glycosides	$FeCl_3$	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
5	Saponins	Foam	-ve	-ve	+ve	-ve	+ve	+ve	+ve	+ve
6	Terpenoids	Libermann-Burchard Test	+ve	+ve	-ve	-ve	+ve	+ve	+ve	-ve
		Salkowski Test	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
7	Protein	Warming Test	+ve	+ve	+ve	+ve	-ve	-ve	-ve	+ve
		Biuret Test	-ve	+ ve	-ve	-ve	-ve	+ve	-ve	-ve
8	Carbohydrate	Molish Reagent Test	+ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve

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9	Phenol	Ferric Chloride	-ve	+ve	+ve	-ve	-ve	-ve	+ve	-ve
10	Amino Acid	Ninhydrine	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
11	Coumarin	Coumarin	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve

Table 3: Preliminary phytochemical tests of MK1 Plant

S. No	**Phyto constituents**	**Chemical Tests**	**MKF**		**MKR**		**MKS**		**MKL**	
			Acetone	**Methanol**	**Acetone**	**Methanol**	**Acetone**	**Methanol**	**Acetone**	**Methanol**
1	Alkaloides	Wagner's Test	-ve	-ve	-ve	+ve	+ve	+ve	-ve	-ve
		Dragendroff's Test	-ve	-ve	-ve	-ve	+ve	+ve	+ve	+ve
		Mayer's Test	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
		Hager's Test	+ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve
2	Flavonoids	Alkaline Test	+ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve
		Lead Acetate Test	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
3	Tannins	Ferric Chloride	-ve	+ve	-ve	+ve	-ve	+ve	-ve	-ve
4	Glycosides	$FeCl_3$	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
5	Saponins	Foam	+ve	+ve	-ve	-ve	-ve	+ve	+ve	-ve
6	Terpenoids	Libermann-Burchard Test	-ve	-ve	+ve	-ve	+ve	+ve	+ve	-ve
		Salkowski Test	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
7	Protein	Warming Test	-ve	+ve	+ve	+ve	-ve	+ve	-ve	+ve
		Biuret Test	-ve	-ve	-ve	-ve	-ve	+ve	-ve	-ve

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8	Carbohydrate	Molish Reagent Test	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
9	Phenol	Ferric Chloride	+ve	+ve	+ve	-ve	-ve	-ve	+ve	-ve
10	Amino Acid	Ninhydrine	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
11	Coumarin	Coumarin	-ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve

Table 4: Preliminary phytochemical tests of MK4 Plant

S. No.	**Phyto constituents**	**Chemical Tests**	**MKL**		**MKS**		**MKF**		**MKR**	
			Acetone	**Methanol**	**Acetone**	**Methanol**	**Acetone**	**Methanol**	**Acetone**	**Methanol**
1	Alkaloides	Wagner's Test	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
		Dragendroff's Test	+ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve
		Mayer's Test	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
		Hager's Test	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
2	Flavonoids	Alkaline Test	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
		Lead Acetate Test	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
3	Tannins	Ferric Chloride	-ve	+ve	+ve	+ve	+ve	-ve	+ve	-ve
4	Glycosides	FeCl3	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
5	Saponins	Foam	-ve	+ve	+ve	-ve	-ve	-ve	+ve	+ve
6	Terpenoids	Libermann-Burchard Test	+ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve
		Salkowski Test	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve

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7	Protein	Warming Test	-ve	+ve	+ve	+ve	-ve	-ve	-ve	+ve
		Biuret Test	-ve	+ve	-ve	-ve	-ve	+ve	-ve	+ve
8	Carbohydrate	Molish Reagent Test	+ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve
9	Phenol	Ferric Chloride	-ve	-ve	+ve	+ve	-ve	-ve	-ve	-ve
10	Amino Acid	Ninhydrine	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
11	Coumarin	Coumarin	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve

Discussion: The phytochemical screening of methanolic & acetonic extracts of fruit, root, stem and leaf of *Murraya Koenigii* collected from Assam, Hisar, Hyderabad and Rudraprayag revealed the presence of Alkaloids, Flavonoids, Glycosides, Protein, Carbohydrate and Coumarin shown in Table 1-4. Methanolic extract of MK3 plant show that Phenol and Amino Acid were not detected in extract of fruit, stem, leaf and root samples. Proteins were detected in stem, leaf, and root extract but not in the fruit extract of *Murraya Koenigii* also Terpenoids and Coumarin were not detected in leaf extract in Table 1.

Acetonic extract of MK3 plant show that Phenol and Amino Acid were not detected in extract of fruit, stem, leaf and root samples and Tannins were detected in leaf extract but not in the fruit, stem and root extract of *Murraya Koenigii* also Terpenoids and Coumarin were not detected in leaf extract Table 1.

Methnolic extract of MK2 plant show that Amino Acid were not detected in extract of fruit, stem, leaf, and root samples and Phenol were not detected in stem, leaf and root extract but in the fruit extract of Murraya koenigii. Terpenoid was detected in fruit and root, extract but not in leaf and stem extract. Saponin was not detected in fruit and leaf extract but in root and stem extract. Tannin was detected in stem extract but not in root, fruit and leaf extract in Table 2.

Acetonic extract of MK2 plant show that Tannins and Amino Acid were not detected in extract of fruit, stem, leaf, and root samples and Phenol were not detected in fruit and root extract but in the leaf and stem extract of *Murraya Koenigii* also Carbohydrate was not detected in fruit and root extract but in leaf and stem extract. Protein was not detected in stem and root extract but in leaf and fruit extract. Terpenoid was detected in fruit, stem and root extract but not in leaf extract in Table 2.

Methanolic extract ofMK1 plant show that Amino Acid was not detected in extract of fruit, stem, leaf and root samples. Phenol was detected in fruit extract but not in the stem, leaf, and root extract of Murraya koenigii. Coumarin was detected in stem, leaf and root extract but not in fruit extract. Terpenoid was detected in stem extract but not in fruit, leaf and root extract. Saponin was detected in fruit and stem extract but not in root and leaf extract. Tannin was detected in fruit, root and stem extract but not in leaf extract in Table 3.

Accetonic extract of MK1 plant show that Tannins and Amino Acid were not detected in extract of fruit, stem, leaf and root samples. Terpenoids, Carbohydrate and Coumarin were detected in leaf, stem and root extract but not in fruit extract. Protein was detected in root extract but not in the leaf, fruit and stem extract of *Murraya Koenigii* also. Phenol was detected in fruit, stem and root extract but not in stem extract. Saponin was detected in leaf and fruit extract but not in stem and root extract in Table 3.

Methanolic extract of MK4 plant show that Amino Acid were not detected in extract of fruit, stem, leaf, and root samples. Phenol was detected in stem extract but not in the fruit, leaf, and root extract of Murraya koenigii. Terpenoid was not detected in fruit extract but in stem, leaf and root extract. Saponin was detected in root and leaf extract but not in fruit and stem extract. Tannin was detected in leaf and stems extract but not in fruit and root extract in Table 4.

Accetonic extract of MK4 plant show that Amino Acid were not detected in extract of fruit, stem, leaf, and root samples. Phenol was detected in stem extract but not in leaf, fruit and root extract. Carbohydrate were detected in leaf, root and stem extract but not in the fruit extract of *Murraya Koenigii* also. Protein was detected in stem extract but not in fruit, leaf and root extract. Saponin was not detected in leaf and fruit extract but in stem and root extract. Tannins were detected in fruit, stem and root extract but not in leaf extract in Table 4.

Conclusion

This study analyze the prelimnary phytochemical screening of Alkaloides, Flavonoids, Tannins, Glycosides, Saponins, Terpenoids, Protein, Carbohydrate, Phenol, Amino Acid and Coumarin compound of *Murraya Koenigii* from different eco-geographical region. This study show that the *Murraya Koenigii* has the richest source of antioxidant such as Alkaloides, Flavonoids, Tannins, Glycosides, Saponins, Terpenoids, Protein, Carbohydrate, Phenol, and Coumarin. But Amino acid wad not found in the Murraya Koenigii. *Murraya Koenigii* plant has used as medicinal plant and food flavoring and spicing condiment.

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