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Phytochemical analysis and percent extractability of *Delonix regia*, *Cassia alata* and *Murraya koenigii* leaf extracts

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Abstract

Medicinal plants have bioactive compounds which are used for curing of various diseases and play an important role in healing. Medicinal plants have antifungal, antibacterial and anti-inflammation activities. The present study involves three different medicinal plants *Delonix regia*, *Cassia alata* and *Murraya koenigii* locally available in campus of COVAS Udgir. The aqueous and methanol leaf extract samples were used for the phytochemical analysis to find out the percent extractability and phytochemical constituents in the plants. The main objective of the research work was to check the presence or absence of the phytochemical constituents in all the selected medicinal plants. The results of the phytochemical analysis of these medicinal plants showed that the glycosides, reducing sugars, flavonoids and alkaloids were found to be present in the mentioned medicinal plants. The pharmaceutical companies now a days are gaining interest to make use of phytoconstituents for curing of various diseases.

Keywords: Medicinal plants, phytochemicals, *Delonix regia*, *Cassia alata*, *Murraya koenigii*

Introduction

Nature has been a source of wide diversity of medicinal plants and mankind has used many species for centuries since time immemorial for their benefit. The medicinal property of the plants has been exploited for the cure of several ailments [4]. There has been an increasing interest worldwide on therapeutic values of natural products from plants due to disenchantment with modern synthetic drug [1]. Herbal medicine has proved efficacious and potent in the treatment of many chronic diseases that orthodox medicine cannot cure. Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions, and to defend against attack from predators such as insects, fungi and herbivorous mammals. Antibiotics were discovered to provide the source for the therapy of microbial infections. Excessive use of antibiotics has become the major factor for the emergence and dissemination of multidrug resistant strains [16]. Numerous aromatic spicy and medicinal plants have been examined for their antioxidant potential [3]. Many plant components are now synthesized and analyzed in large laboratories, for example vincristine (antitumor drug) and ephedrine (bronchodilator) used to decrease respiratory congestion were well originally discovered through research on medicinal plants [9]. In general people with low income such as farmers, people of small isolated villages and native communities use folk medicine for the treatment of common infections [8]. Infectious diseases caused by these multidrug resistant strains remain the leading cause of death. Thus people are turning their attention to alternative novel antimicrobial agents to combat such pathogens [5, 10].

Phytochemicals are biologically active compounds derived from plant physiological processes and are, responsible for conferring colour, flavor, smell, texture and several biological properties including antimicrobial property to the plants [11]. India is rich in all the three levels of bio diversities including species diversity, genetic diversity and habitat diversity. Therefore, after search through wide literature for medicinal plants bearing antimicrobial properties the plants *Delonix regia*, *Cassia alata*, and *Murraya koenigii* plants were selected to further screen their phytochemical properties. The aim to analyze the phytochemical properties of the concerned plants was to validate their use in folk medicine. The perception was also that the isolation and characterization of the phytoconstituents present if any will definitely give scope for the discovery of novel active compounds.

Material and Methods

Collection and identification of plant sample

The three local plants *Delonix regia*, *Cassia alata*, and *Murraya koenigii* were identified for the study and were procured from the campus of College of Veterinary and Animal Sciences, Udgir and suburban areas around Udgir. The plants were duly authenticated by the botanist from the Department of Botany, Shivaji College, Udgir of Maharashtra State.

Preparation of the extracts

The leaves of the plant were dried under shade and the powder of the dried leaves was prepared by an electrical grinder. The dried powder was passed through sieve to obtain the fine powder. The fine leaf powder was stored in airtight glass bottles, which were kept in the refrigerator. Fifty grams of the powder was taken in two separate 500ml conical flask. Two hundred milliliters of distilled water and methanol were added to these two flasks. The flasks were stoppered tightly and were kept in refrigerator for 48 hrs. of maceration process and flasks were shaken intermittently during the maceration period. After the maceration process, the content was filtered through muslin cloth and the filtrate so obtained was once again filtered through what man filter paper no 1. The filtrate was transferred to sterilized evaporating bowl, which was then placed under the fan for evaporation of the solvent so as to make the extract dry as much dry as possible.

Determination of extractability percentage

The extractability of each of these extracts were determined as per the method suggested by the Rosenthaler (1930) ^[12] using following formula

$$\% \text{ Extractability} = \frac{\text{weight of extract}}{\text{Powder used}} \times 100$$

Phytochemical screening: The preliminary qualitative phytochemical analysis was done as per method described by Rosenthaler (1930)¹² to detect presence or absence of various phytoconstituents of plant extracts.

1. Test for detection of alkaloids

A small amount of extract was taken in a test tube and added with 5ml of 1.5% HCL and then filtered. The filtrate was used for the presence of alkaloids. A few drops of each of the following reagents were added to the filtrate and mixed well, appearance of turbidity or any changes in colour to the test indicate the presence of alkaloids.

a. Wagner's reagent test

The reagent was prepared by dissolving iodine 1.27G of iodine and 2G of potassium iodide in 5ml of distilled water and diluted to 100ml. The little amount of extract filtrate was added to this reagent, appearance of brown to flocculent precipitation indicates the presence of alkaloids.

2. Test for Detection of Glycoside

a. Folin Wu copper reagent Test

A little amount of the extract was added to few drops of folium copper reagent, the development of red colour gives positive reaction for glycoside.

b. Fehling reagent's Test

Fehling - A solution: Copper sulphate (34.66 gm) was dissolved in distilled water and volume made upto 500 ml. Fehling - B solution: Potassium sodium tartarate (173 gm)

and sodium hydroxide (50 gm) were dissolved in water and volume made upto 500 ml.

Few mg of extract was added with 0.5ml of Fehling reagent's A and B and 2ml of 10% sodium hydroxide solution. The mixture was heated on water bath for 10 minutes. Appearance of red precipitate revealed presence of glycoside, to clear the solution few drops of HCL was added and boiled for five minutes. The Fehling reagents was again added to record any further reduction, which indicates the test positive for Glycoside.

3. Test for Detection of proteins

a. Biuret Test

A few amount of residue were taken in water and 1 ml of 1% solution of sodium hydroxide was added followed by a drop of 1% solution of copper sulphate. Violet pink color development indicates positive test for proteins.

4. Test for Detection of reducing sugar

a. Benedict's reagent test

The extract was added with Benedicts reagent in equal amount and mixture was heated for 2 minutes, appearance of brown to red colour indicates presence of reducing.

b. Folin Wu copper reagent test

Small quantity of the extract was added with few drops of Folin Wu copper reagent, the development of red colour indicates presence of reducing sugar

5. Test for Detection of Tannins

A little quantity of aqueous extract taken in a test tube was warmed and filtered. Tests were carried out with the filtrate using following reagent.

a. Lead acetate Test

Few drops of 5% lead acetate solution were added to the filtrate formation of precipitation indicates the presence of tannins.

b. Ferric chloride Test

Few drops of ferric chloride were added to the little amount of the filtrate development of green colour indicates presence of tannins.

6. Test for resins

The alcoholic extract was dissolved in alcohol. To this, a few drops of water were added. The appearance of turbidity was considered as a positive test.

7. Test for phytosterols

a. Salkowski's Test

A small amount of extract was taken in 2 ml of chloroform and sulphuric acid was added alongside of test tube and test tube was shaken. Red color development in the chloroform layer and greenish yellow fluorescence in the lower layer indicates presence of sterols

8. Test for Anthraquinones

a. Bentrager's Test

Small amount of the extract was added with 5ml of 10% sulfuric acid and boiled for few minutes then, filtered immediately. The filtrate was cooled and shaken with Benzene, the benzene layer was separated and also shaken with half of its volume of 10% ammonia, the ammonical layer acquiring pink colour indicate the presence of anthraquinones.

9. Test for Saponins

The extract (50 mg) was taken in stoppered test tube and

finally diluted upto 20 ml by adding distilled water. The tube was shaken for 15 min and observed for formation of foam. A two centimeter foam layer indicates positive test.

10. Test for flavonoids

A small quantity of residue was dissolved in 5 ml of ethanol (95%) and treated with a few drops of concentrated hydrochloric acid and 0.5 gm of magnesium metal turnings. Development of either pink or red color indicates presence of flavonoids.

Result and Discussion

The physical properties (colour and consistency) and percent extractability of different plant leaf extracts is as shown in table 1. The colour of aqueous leaf extract of *Delonix regia*, *Cassia alata* and *Murraya koenigii* was bright reddish brown, brown and brown respectively. The colour of methanol leaf extract of *Delonix regia*, *Cassia alata* and *Murraya koenigii* was green, greenish brown and dark green. The aqueous leaf extract of *Delonix regia*, *Cassia alata* and *Murraya koenigii* was solid, semisolid and sticky in consistency while the methanol leaf extract was semisolid in consistency of *Delonix regia*, *Cassia alata* and *Murraya koenigii*. The percent extractability of *Delonix regia*, *Cassia alata* and *Murraya koenigii* leaf powder in aqueous and methanol solvent varies between 2.3 to 6.3. The percent extractability was maximum (6.3) for *Cassia alata* aqueous and lower (2.3) for leaf extract *Delonix regia* aqueous.

In the present studies phytochemical screening of three plants of *Delonix regia*, *Cassia alata* and *Murraya koenigii* were done. The phytochemical analysis of three plant is presented

in tables 2 and 3. The result reveals that some of the phytochemicals analyzed were present in the extracts of all the plants.

On the ten phytochemicals screened, glycosides, flavonoids and phenols were present commonly in all the studied plant. From *Delonix regia* extracts (Table 2 and 3) alkaloids, glycosides, Saponins, phytosterols, tannins and flavonoids were present in distilled water, alkaloids, glycosides and flavonoids were present in methanol solvent and this results is reported by Sama *et al* (2012) [14]. In *Cassia alata* extracts alkaloids, saponins, tannins, flavonoids and Anthraquinones were present in distilled water and alkaloids, glycosides, saponins, phytosterols, tannins, flavonoids, resins, Anthraquinones and reducing sugar present in methanol solvent, were founded by Doughari J.H and Oka for B., (2007) [6]. Senthilkumar *et al.* (2013) [15]. Ehiowemwenguan *et al.*, (2014) [7]. In *Murraya koenigii* extracts (table 2 and 3) Saponins, proteins, phytoesterols, flavonoids and reducing sugar were present in distilled water and alkaloids, proteins, phytoesterols, tannins, flavonoids and reducing sugar present in methanol solvent shown in table 2 and 3. This result is supported by Argal *et al.*, (2011) [2], Kumar *et al.*, (2007) [11], Salomi M.V., and Manimekalai R. (2016) [13].

In conclusion, the selected three medicinal plant in this study consist of many useful phytochemical compounds having important biological properties. The result of this study would lead to find out some compounds which are very useful for the manufacturing of new drugs. The previous phytochemical analysis and present studies show nearly the similar results due to the presence of phytochemical constituents.

Table 1: Percent extractability of different plant extracts

S. No	Content	<i>Delonix regia</i>		<i>Cassia alata</i>		<i>Murraya koenigii</i>	
		Aqueous	Methanol	Aqueous	Methanol	Aqueous	Methanol
1	Solvent used	Aqueous	Methanol	Aqueous	Methanol	Aqueous	Methanol
2	Quantity of powder	50 g	50 g	50 g	50 g	50 g	50 g
3	Color	Bright reddish brown	Green	Brown	Greenish brown	Brown	Dark green
4	Consistency	Solid	Semisolid	Semisolid	Semisolid	Sticky	Semisolid
5	Extractability %	2.3	3.1	6.3	4.30	6.16	4.16

Table 2: Phytochemical screening of aqueous leaf extract of *Delonix regia*, *Cassia alata* and *Murraya koenigii*

S. No	Phytochemicals	Aqueous plant extracts		
		<i>Delonix regia</i>	<i>Cassia alata</i>	<i>Murraya koenigii</i>
1.	Alkaloids	+	+	-
2.	Glycosides	+	-	-
3.	Saponins	+	+	+
4.	Proteins	-	-	+
5.	Phytosterols	+	-	+
6.	Tannins	+	+	-
7.	Flavonoids	+	+	+
8.	Resins	-	-	-
9.	Anthraquinones	-	+	-
10.	Reducing sugar	-	-	+

+: present, - absence

Table 3: Phytochemical screening of methanol leaf extract of *Delonix regia*, *Cassia alata* and *Murraya koenigii*

S. No	Phytochemicals	Methanol plant extracts		
		<i>Delonix regia</i>	<i>Cassia alata</i>	<i>Murraya koenigii</i>
1.	Alkaloids	+	+	+
2.	Glycosides	-	+	-
3.	Saponins	-	+	-
4.	Proteins	-	-	+
5.	Phytosterols	+	+	+
6.	Tannins	-	+	+
7.	Flavonoids	+	+	+
8.	Resins	-	+	-
9.	Anthraquinones	-	+	-
10.	Reducing sugar	-	+	+

+: present, - absence

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