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Research Article

In Vivo and *In Vitro* Phytochemical Screening, Comparative Analysis and Sub Culturing Effect of Calotropisprocera

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Abstract

Calotropisprocera (Asclepiadaceae) is commonly called "madar" [1-3]. It is a useful medicinal plant. C. procera is an effective source of traditional and modern medicine, used in primary health care [4-11]. Plants are the richest source of bioactive phytochemicals on earth. The active metabolites or the Phytochemicals from the medicinal plants are used for developing novel and biodegradable drugs [12-17]. C. procerais of great medicinal importance and is used to treat fever, indigestion, cold, cough, cardio tonic, asthma, scabies etc.

Phytochemical activities of chloroform extracts, obtained from the leaves of C.procera, were investigated in an attempt to evaluate its beneficial aspects including its therapeutic potential. The phytochemical screening reveals the presence of tannins, steroids, and coumarin, in a very high amount in chloroform extracts from *in vitro* cultures and alkaloids, flavonoids, phenolics, and cardiac glycosides are found moderately high concentrations when extracts were taken from *in vivo* explants. Extracts were also taken from various stage subcultures and their results were also analyzed and compared.

The result of this study validates the significant similarity between the chloroform extracts obtained from the leaves of C.procera from *in vivo* source and *in vitro* source. Attempts are also made to emphasize on the fact that phytochemical extract of the leaf can be used for treatment of various ailments.

Keywords: Calotropisprocera; Phytochemicals; Chloroform extracts

Introduction

Phytochemicals are non-nutritive plant chemicals performing protective or preventive functions. Plants produce them to protect themselves but recent researches have demonstrated that many phytochemicals can protect humans too against various diseases [18-21]. As a matter of fact, plants producing secondary metabolites, such as alkaloids, tannins, glycosides, and contain minerals, possess therapeutic properties [19].

C. procera belongs to family Asclepiadaceae and is used therapeutically, to treat boils, wounds with infections and other skin problems such as parasitic skin infections in animals. We also obtain ash for making gun powder. The plant latex can be processed and used to treat baldness, hair fall, tooth aches, joints swellings and paralysis. It is antihelmintic and used as an expectorant. The roots are potential laxatives. Traditionally, the dried roots are powdered and used to cure various chronic diseases such as bronchitis, asthma, leprosy, eczema and elephantiasis, hepatic and splenic enlargement to cite a few [22-25].

The milky juice is regarded as a purgative and the flowers are considered to improve digestion [26,27]. The latex that we get from the leaves of C. procera is processed and used in commercial preparation of eye tonic [28,29]. The juice also induces abortion in women and to remove hair from the hides. It founds use in backache and joint pains. The warm leaves also reduce stomachache if tied around. Inhaling burnt leaves serve to cure headache. Leaf latex when applied on fresh cut, stops the bleeding immediately [30,31]. Various heart stimulant alkaloids such as calotropin, calotaxein and uskerin are also found (Tables 1 and 2). Various traditional medicine practitioners in Gwari communities use the extract for the treatment of skin diseases caused due to ring worms [32].

This paper reports on the phytochemical properties of C. procera and an attempt has been made to investigate the various phytochemicals present and studied the sub culturing effect on the phytochemicals extracted from *in vitro* cultures that can be taken up for further research in the field of drug designing and discovery [35].

Materials and Methods

Chemical reagents used

Chloroform, ethanol, sulphuric acid, Mayer's, Hagner's, and Wagner's reagents, tannic acid solution, HCl, Dagendroff's reagent, NaOH(aq.), acetic anhydride, lead acetate, magnesium and benzene [36,45].

Fresh leaves of C. procera are collected from Noida, Uttar Pradesh. Also for *in vitro* analysis, leaves are taken from C. procera species cultured in Applied Plant Biotechnology Research lab of Amity institute of Biotechnology, AUUP. The plant parts are dried under the sun and then pulverized into coarse powder using mortar

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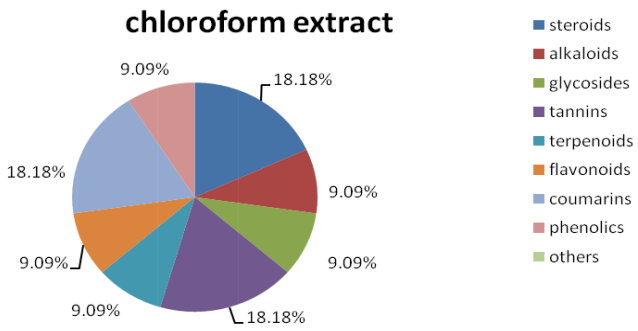

Figure 1: According to the pie chart, in chloroform extract the maximum result was observed for steroids, tannins, and coumarins whereas other phytochemicals were observed in the fewer amounts.

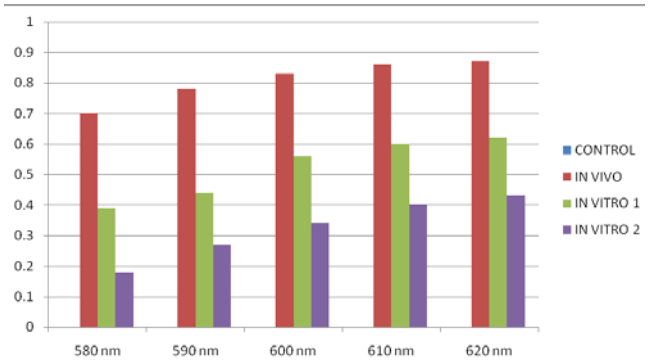

Figure 2: Plot between incident wavelength and optical density values.

and pestle. The powdered samples are then sieved and stored in polythene bags until they are further used [39].

The leaf powder is weighed 5g and kept in a test tube in contact with chloroform for 48hr, with vigorous shaking at regular intervals. The material is filtered at first with muslin cloth and then with filter paper. The filtrate is then collected and dried in a water bath till we observe no further reduction in the mass of extract. Dried extract is weighed and packed in an air tight container [41].

The dry latex of Calotropis leaves is very difficult to dissolve. This happens because plant latex contains tannins, alkaloids, gums, proteins, polysaccharides and many others. There are both polar and non-polar types of constituents in dry latex of plant therefore for dissolving both polar and non-polar solvents were used for complete dissolution of the dry latex. The toluene (moderately polar) was used to dissolve the moderately polar molecules. Then the acetic acid (highly polar solvent) was taken to dissolve the highly polar molecules, if any. Hexane (non-polar) was used keeping in mind to dissolve the non-polar molecules that were bonded. Lastly the HCl was used to dissolve the brownish particle like dirt that gave woody appearance. The dry latex was dissolved in a test tube with 3ml toluene, 2ml acetic acid, 3ml chloroform, and 2ml hexane. This gave a slightly detergent like appearance in the test tube with lots of very small bubbles [44]. After supplementation of HCl (1ml) gave mixture separated into 2 layers where 1st Upper layer is obtained as a clear solution & 2nd lower layer is of light bluish solution. Finally the supernatant were used for further phytochemicals analysis.

Tests done for the analysis

Test for steroids

- 1 ml of the extract was dissolved in 10ml of the chloroform and equal volume of sulphuric acid was added by the sides of the test tube.
- If the upper layer turns red and sulphuric acid layer showed yellow with green fluorescence.
- This indicates the presence of steroids

Test for alkaloids

- 5 ml of extract was added to 2ml of HCL, to this acidic medium 1ml of Dagendroff's reagent was added.
- An orange or red precipitate produced immediately indicates the presence of alkaloids.

Biuret test for protein

- Aqueous sample treated with an equal volume of 1% strong base (sodium or potassium hydroxide)
- Followed by few drops of aqueous copper sulphate (II).

Test for coumarin

- To 2ml of test solution, a few drops of alcoholic sodium hydroxide were added.
- Appearance of yellow colour indicates the presence of coumarin.

Test for reducing sugar

- Mix 15ml of Fehling solution A with 15ml of fehling solution B, add 2ml of this solution to an empty test tube.
- Add 3 drops of this compound to be tested, place tube in a water bath at 60°c.

Test for terpenoids

- 1ml of extract was placed into the test tube and added 0.4ml of chloroform, 0.6ml of concentrated sulphuric acid was poured gently into the tube at an inclined portion.
- A reddish brown coloration was indicative of the presence of terpenoids

Test for glycosides

- 1ml of concentrated sulphuric acid gently poured on the walls of an incline test tubes containing 1ml of plant filtrate.
- Dropwise was added 10% ferric chloride solution and observe for a brown, violet or greenish ring.

Test for flavonoid

- 1ml of 10% NaOH was mixed with 1 ml of filtrates, shaken vigorously and observe for the development of yellow colouration
- Test for tannins
- 1ml of filtrate was added to 1ml of distilled water in a test tube.

Figure 3: (A) For C. procera grown in field (*in vivo*), (B) For C. procera cultured *in vitro* (First subculture), (C) For C. procera cultured *in vitro* (Second subculture).

- A few drops of 10% ferric chloride was added and observed for brownish green or blue black colouration.

Results

A variety of phytochemical tests were performed using Calotropisprocera leaves of external or field grown source, and *in vitro* cultured sources. These tests were to test the presence of steroid, alkaloid, protein, coumarin, reducing sugar, terpenoids, glycosides, flavonoids and tannins. A few different results were obtained for leaves when extracting reagent chloroform was used.

According to the pie chart, in chloroform extract the maximum result was observed for steroids, tannins, and coumarins whereas other phytochemicals were observed in the fewer amounts (Figures 1-3).

A graph was plotted between incident wavelength and optical density values for control sample, sample obtained from external or field source, and those obtained after first and second subcultures respectively. From the plot it has been observed that in all the cases, except in the control case, maximum absorbance or OD value was at 620nm that indicates the maximum presence of closed ring aromatic compounds in the test samples, be it from any source. These are terpenoids, steroids, tannins e.t.c. Also an increasing trend of absorbance is observed as we increase the incident wavelength upto a certain value of incident wavelength. Hence we can conclude from here that a similar kind of increasing trend, however by different amounts of absorbance in individual case, is observed in all the cases

Table 1: Tests done for the analysis.

	1ml of the extract was dissolved in 10ml of the chloroform and equal volume of sulphuric acid was added by the sides of the test tube.
	If the upper layer turns red and sulphuric acid layer showed yellow with green fluorescence.
	This indicates the presence of steroids
Test for alkaloids	5ml of extract was added to 2ml of HCL, to this acidic medium 1ml of Dragendorff's reagent was added.
	An orange or red precipitate produced immediately indicates the presence of alkaloids.
Biuret test for protein	Aqueous sample treated with an equal volume of 1% strong base (sodium or potassium hydroxide)
	Followed by few drops of aqueous copper sulphate (II).
Test for coumarin	To 2ml of test solution, a few drops of alcoholic sodium hydroxide were added.
	Appearance of yellow colour indicates the presence of coumarin.
Test for reducing sugar	Mix 15ml of Fehling solution A with 15ml of fehling solution B, add 2ml of this solution to an empty test tube.
	Add 3 drops of this compound to be tested, place tube in a water bath at 60°c.
Test for terpenoids	1ml of extract was placed into the test tube and added 0.4ml of chloroform, 0.6ml of concentrated sulphuric acid was poured gently into the tube at an inclined portion.
	A reddish brown coloration was indicative of the presence of terpenoids
Test for glycosides	1ml of concentrated sulphuric acid gently poured on the walls of an incline test tubes containing 1ml of plant filtrate.
	Dropwise was added 10% ferric chloride solution and observe for a brown, violet or greenish ring.
Test for flavonoid	1ml of 10% NaOH was mixed with 1ml of filtrates, shaken vigorously and observe for the development of yellow colouration
Test for tannins	1ml of filtrate was added to 1ml of distilled water in a test tube.
	A few drops of 10% ferric chloride was added and observed for brownish green or blue black colouration.

Table 2: A variety of phytochemical tests were performed using Calotropisprocera leaves of external or field grown source, and *in vitro* cultured sources. These tests were to test the presence of steroid, alkaloid, protein, coumarin, reducing sugar, terpenoids, glycosides, flavonoids and tannins. A few different results were obtained for leaves when extracting reagent chloroform was used.

Phytochemicals	External	First SC	Second SC
Flavonoids	+	+	+
Glycosides	+	++	+
Terpenoids	+	-	-
Tannins	++	++	+
Alkaloids	+	+	+
Coumarins	++	+	+
Reducing sugar	-	-	-
Phenolics	+	+	+
Saponins	-	-	-
Proteins	-	-	-
Steroids	++	+	++

where leaf extracts were taken. Hence the graph verdicts considerable extent of similarity between field grown C. procera and *in vitro* cultured C. proceraleaves.

Discussion and Conclusion

Calotropisprocera is tested for presence or absence of certain biomolecules, and hence a qualitative testing was performed to observe best results. There has been a considerable level of similarity in the results when the field grown and *in vitro* cultured C. procera plants are compared. They have been tested for tannins, flavonoids, Terpenes, steroids, proteins, glycosides and other such compounds. Solvents or extracting agent chloroform was used in order to get the maximum efficiency of extraction. Chloroform was taken as solvent in order to get maximum positive results. The findings can further be used in drug development, cosmetics, food industry and for other analytical purposes.

The phytochemicals that are obtained can further be used to make drugs so as to cure the diseases or supplement the deficiency of any nutrient in the body, and find wide range of applications in cosmetics industry. Also on observing the considerable degree of similarity between the *in vivo*, First and second subculture leaves' extracts, we can hence conclude that C. procera can be cultured *in vitro* and its potential benefits can be utilized for human welfare.

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