Annals of Phytomedicine 3(2): 35-39, 2014

Journal homepage: www.ukaazpublications.com

ANNALS OF PHYTOMEDICINE
An International Journal

ISSN 2393-9885

Monitoring *in vitro* phytochemical analysis of some diabetic plants and its utilization

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Received October 21, 2014: Revised November 13, 2014: Accepted November 15, 2014: Published online December 30, 2014

Abstract

North eastern terai region in Uttar Pradesh, India is known for its rich biological diversity. The most commonly employed species, used for the treatment of diabetes such as *Casearia tomentosa* Roxb. (root), *Cassia fistula* Linn. (flowers), *Catharanthus roseus* (L.) G. Don (flowers), *Coccinia grandis* (L.) Voigt. (fruits), *Dillenia indica* Linn. (leaves), *Dillenia pentagyna* Roxb. (fruits), *Ficus benghalensis* Linn. (bark), and *Momordica charantia* Linn. (fruits) were selected for phytochemical analysis. The aim of the present study was to investigate the presence of phytochemicals and to determine the ascorbic acid, total phenolic and flavonoid contents of the selected plants. Total phenolic contents obtained were 9.2mg/gm, 9.4mg/gm, 5.8mg/gm, 14.6mg/gm, 14.8mg/gm, 20.4mg/gm, 12.8mg/gm, and 35.3mg/gm of the extracts, total flavonoid contents obtained were 4.2mg/gm, 18.3mg/gm, 4.4mg/gm, 6mg/gm, 21.4mg/gm, 18mg/gm, 3.1mg/gm, 14.4mg/gm of the extracts and ascorbic acid content obtained were 8.21µg/gm fw, 4.82µg/gm fw, 4.42µg/gm fw, 5.12µg/gm fw, 2.42µg/gm fw, 18.21µg/gm fw,2.12µg/gm fw, 7.41µg/gm fw of the extract for the selected plants, respectively. An ethnobotanical field study reveals that the ethnic people have considerable traditional knowledge of these plants and their utilization.

Key words: Phytochemicals, diabetes, ethnobotanical field, traditional knowledge.

1. Introduction

Plant provides food, raw materials for medicine and various other requirements for the very existence of life from the origin of human beings (Subramoniam, 2014). The plant kingdom is a treasure house of potential drugs and in the recent years, there has been an increasing awareness about the importance of medicinal plants (Yadav and Agarwala, 2011). Diabetes mellitus (DM) remains a global major health problem in the world over with the tropics inclusive. In the past decade, the United States have recorded a 33% rise in the cases of diabetes.The two types of diabetes are referred to as type 1 and type 2. Former names for these conditions were insulin-dependent and non-insulin-dependent diabetes, or juvenile onset and adult onset diabetes. DM is a chronic disorder in humans and responsible for different complications and also causes mortality and morbidity. Fruits are rich in antioxidants that help in lowering incidence of degenerative diseases such as cancer, arthritis, arteriosclerosis, heart disease, inflammation, brain dysfunction and acceleration of the ageing process (Feskanich *et al*., 2000; Gordon, 1996; Halliwell, 1996). *Momordica charantia* Linn., also known as bitter melon, bitter gourd, or balsam pear, is a plant widely cultivated in many tropical and subtropical regions of the world and is frequently used in South Asia and the Orient as a food stuff and medicinal plant (Sharma *et al*., 2014). According to World Health Organization (WHO), medicinal plants would be the best source to obtain variety of drugs. About 80% of individuals from developed countries use traditional medicines, which has compounds derived from medicinal plants. However, such plants should be investigated to better understand their properties, safety, and efficiency (Kumar and Selvam, 2009). Medicinal plants contain some organic compounds which provide definite physiological action on the human body and these bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids (Edoga *et al*., 2005). Numerous plant foods or physiologically active ingredients derived from plants have been investigated for their role in disease prevention and health (Pushpangadan *et al.,* 2014).

Plant products have been part of phytomedicines since time immemorial. This can be derived from barks, leaves, flowers, roots, fruits, seeds (Criagg and David, 2001). Knowledge of the chemical constituents of plants is desirable because such information will be value for synthesis of complex chemical substances (Mojab *et al*., 2003; Parekh and Chanda, 2007; Parekh and Chanda, 2009). Plants and their products have been the reliable remedy for humankind since ancient times for various ailments (Srikanth *et al.,* 2014). Our findings provided evidence that crude aqueous and organic solvent extracts of these tested plants contain medicinally important bioactive compounds and it justifies their use in the traditional medicines for the treatment of different diseases. In the present work, qualitative and quantitative estimations were carried out in different parts of eight plants, *Casearia tomentosa* Roxb., *Cassia fistula* Linn., *Catharanthus roseus* (L.) G. Don, *Coccinia grandis* (L.) Voigt., *Dillenia indica* Linn., *Dillenia pentagyna* Roxb., *Ficus benghalensis* Linn., and *Momordica charantia* Linn. of Northeastern terai region in Uttar Pradesh.

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2. Material and Methods

2.1 Collection of plant material

The present work is the outcome of ethnobotanical field survey of one consecutive year (March 2012 to March 2013) from different tribal villages in Shrawsti district of Uttar Pradesh. Sample parts of eight diabetic plants,*Casearia tomentosa* (root),*Cassia fistula* (flowers),*Catharanthus roseus* (flowers),*Coccinia grandis* (fruits), *Dillenia indica* (leaves), *Dillenia pentagyna* (fruits),*Ficus benghalensis*(stem and root),and *Momordica charantia* (fruits) were collected from Bhinga forest range and Shohelwa forest range. The plant materials were taxonomically identified by the Division of Ethnobotany and Ecology, National Botanical Research Institute, Lucknow. The sample part was shock-frozen in liquid nitrogen and kept at – 20 ºC before freeze drying (Christ; Osterode, Germany). Freeze-dried samples were ground for subsequent extraction and compound analysis.

2.2 Preparation of extracts

Crude plant extract was prepared by Rota vapour extraction method. About 200gm of dry plant material kept in 1 litre conical flask then add 500ml ethanol completely deep for 24 hours. A solvent was separated from sample then for extraction process, solvent is kept in RBF of Rota vapour. After that the extract was taken in a beaker and kept on hot plate and heated at 30-40ºC till all the solvent got evaporated. Dried extract was kept in refrigerator at 4ºC for their future use in phytochemical analysis.

2.3 Chemicals

All the chemicals and solvents used were of analytical grade (AR), procured from Sisco Research Lab., Himedia, Rankem, Glaxo, SD Fine Chemicals and other standard firms. Biochemical grade fine chemicals were purchased from Sigma Chemicals Co., USA, Germany and J.T. Baker, USA.

2.4 Qualitative phytochemical analysis

The extract was tested for the presence of bioactive compounds by using following standard methods (Sofowra, 1993; Trease and Evans, 1989; Harborne, 1973).

Test for proteins: (Millon's test)

Crude extract when mixed with 2ml of Millon's reagent, white precipitate appeared which turned red upon gentle heating that confirmed the presence of protein.

Test for carbohydrates: (Benedict's test)

Crude extract when mixed with 2ml of Benedict's reagent and boiled, a reddish brown precipitate formed which indicated the presence of the carbohydrates.

Test for phenols and tannins

Crude extract was mixed with 2ml of 2% solution of $FeCl_3$. A blue-green or black coloration indicated the presence of phenols and tannins.

Test for flavonoids: (Shinoda test)

Crude extract was mixed with few fragments of magnesium ribbon and concentrated HCl was added drop wise. Pink scarlet colour appeared after few minutes which indicated the presence of flavonoids.

Test for saponins

Crude extract was mixed with 5ml of distilled water in a test tube and it was shaken vigorously. The formation of stable foam was taken as an indication for the presence of saponins.

Test for alkaloids

Crude extract was mixed with 2ml of 1% HCl and heated gently. Mayer's reagents was then added to the mixture. Turbidity of the resulting precipitate was taken as evidence for the presence of alkaloids.

2.5 Quantitative phytochemical analysis

2.5.1 Total phenolic content

The amount of phenol in the aqueous extract was determined by Folin-Ciocalteu reagent method with some modifications. 2.5ml of 10% Folin-Ciocalteu reagent and 2ml of 2% solution of Na_2CO_3 were added to 1ml of plant extract. The resulting mixture was incubated for 15 minutes at room temperature. The absorbance of the sample was measured at 765nm. Gallic acid was used as standard (1mg/ml). All the tests were performed in triplicates. The results were determined from the standard curve and were expressed as gallic acid equivalent(mg/g of extracted compound) (Aiyegroro and Okoh, 2010).

2.5.2 Total flavonoid content

Aluminium chloride colorimetric method was used with some modifications to determine flavonoid content. 1ml of sample plant extract was mixed with 3ml of methanol, 0.2ml of 10% aluminium chloride, 0.2ml of 1M potassium acetate and 5.6ml of distilled water and remains at room temperature for 30 minutes. The absorbance was measured at 420nm. Quercetin was used as standard (1mg/ml). All the tests were performed in triplicates. Flavonoid contents were determined from the standard curve and were expressed as quercetin equivalent (mg/g of extracted compound) (Aiyegroro and Okoh, 2010).

2.5.3 Ascorbic acid content

Ascorbic acid content in the roots (50mg each), leaves (200 mg each), and flowers (100mg each) of the plant was estimated by the method of Keller and Schwager (1977).

3. Results and Discussion

The results of the phytochemical screening of the plant extracts in ethanolic extractions have shown a notable of phytochemical compounds. From the Table 1, it could be seen that, proteins, phenols and tannins, flavonoids and saponins were present in all the plants. Alkaloids were absent only in the bark of *Ficus benghalensis* while, carbohydrates were absent in the flowers of *Cassia fistula*.*Casearia tomentosa, Catharanthus roseus, Coccinia grandis, Dillenia indica, Dillenia pentagyna, and Momordica charantia* species are most important plant for secondary metabolites in between selected plants.Total phenolic contents obtained were 9.2mg/gm, 9.4mg/gm, 5.8mg/gm, 14.6mg/gm, 14.8mg/gm, 20.4mg/gm, 12.8mg/gm, and 35.3mg/gm of the extract. Total flavonoid contents obtained were 4.2mg/gm, 18.3mg/gm, 4.4mg/gm, 6mg/gm, 21.4mg/gm, 18mg/gm, 3.1mg/gm, and 14.4mg/gm of the extract and ascorbic acid content obtained were 8.21µg/gm fw, 4.82µg/gm fw, 4.42µg/gm fw, 5.12µg/gm fw, 2.42µg/gm fw, 18.21µg/gm fw, 2.12µg/gm fw, 7.41µg/gm fw of the extract for the selected plants, respectively. We find that only *Dillenia pentagyna* fruits have more amounts of phenolic content, flavonoid, and ascorbic acid between selected plants.Phytochemical screening is of paramount importance in identifying new source of therapeutically and industrially valuable compounds having medicinal significance, to make the best and judicious use of available natural wealth (Arvind *et al*., 2010). Screening of the plant extracts revealed the presence of phytochemicals such as phenols, tannins, flavonoids, saponins, steroids, and alkaloids. The phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites (Singh *et al*., 2007). The

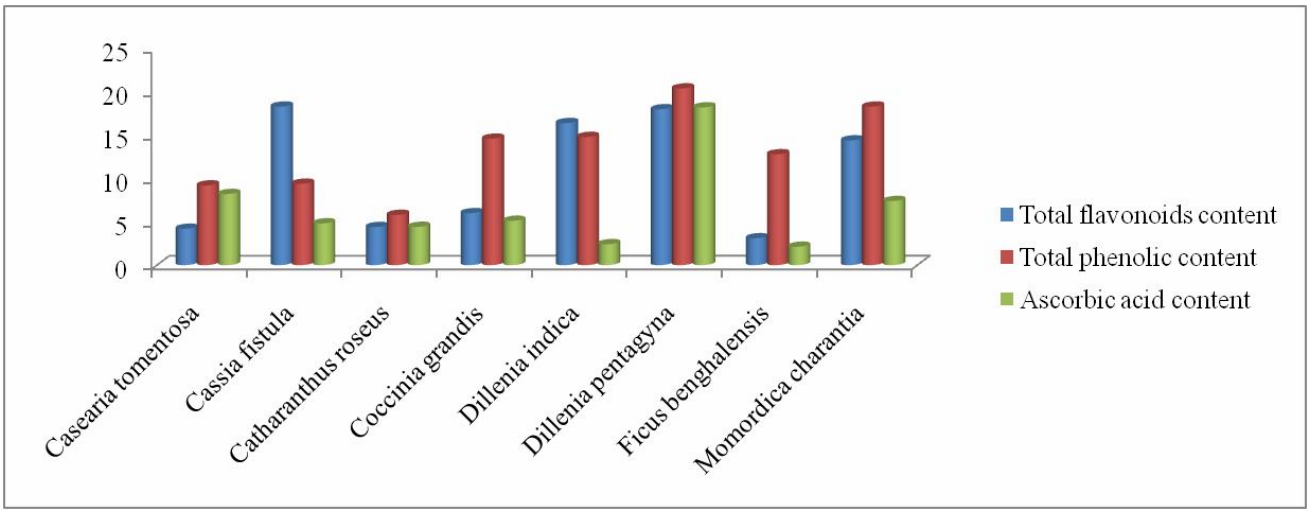
enzyme inhibitory effects correlates with the medicinal properties and phytochemical components such as phenolic compounds, alkaloids, saponins, *etc*. (Urooj *et al*., 2014). Nutraceuticals are plant products with nutritional and medicinal value. At present time, nutraceutical plants are in great demand, considering their safety and health benefits, particularly, the developed world (Subramoniam, 2014).

Phenolics have attracted a great attention in relation to their potential for beneficial effects on health. Over the last few years, several experimental studies have revealed biological and pharmacological properties of phenolics compounds, especially their antimicrobial activity (Narayana *et al*., 2001), antiviral, anti-inflammatory and cytotoxic activity (Chhabra *et al*., 1984). Phenolics are active in curing kidney and stomach problems as well as helpful as anti-inflammatory in action (Zhu *et al*., 1997)**.** Tannins decrease the bacterial proliferation by blocking key enzymes at microbial metabolism (Geidam *et al*., 2007). Tannins play an important role such as potent antioxidant (Trease and Evans, 1983)**.** Alkaloids have been associated with medicinal uses for centuries and one of their common biological properties is their cytotoxicity (Nobori *et al*., 1994). The juice of *Dillenia indica* leaves, bark, and fruits are mixed and given orally (5-15ml, 2 to 5 times daily) in the treatment of cancer and diarrhoea (Sharma *et al*., 2001)**.** Antidiabetic and hypolipidemic activities of bioactive fraction of *D. indica* methanolic extract (fractioned with ethyl acetate) was analysed in experimental diabetic Wistar rats. Type 1 and Type 2 diabetes were induced using streptozotocin and nicotinamide as a standard (Intraperitoneally) and treated by giving fraction orally (Gandhi and Mehta, 2013). The potential role of dietary antioxidants, such as ascorbic acid, tocopherol, β-carotene, *etc.* to reduce the activity of free radical-induced reactions, has drawn increasing attention (McDermott and Powell, 1996)**.** Ethanol extract of *D. pentagyna* showed antitumor activity against ascites Dalton's lymphoma, and concentration of iridoids and high concentration of tannins in *D. pentagyna* (Rosangkima *et al*., 2010)**.**Present investigation reported that this plant is ware house of chemo-diversity which will be useful in screening for medicines like steroids, alkaloids, phenolics, flavonoids and some other chemicals. Antidiabetic activity concludes that this plant is an important aspect of controlling elevated blood sugar in patients with diabetes.The results are encouraging but scientific scrutiny is absolutely necessary before being put in practice.

Table 1: Qualitative phytochemical analysis of ethanolic extract and utilization of eight diabetic plants studied

S.No.	Plants / Family	Protein	Carbohydrate	Phenol / Tanin	Flavonoids	Saponin	Alkaloids	Direct utilization by tribes for the treatment of Diabetes.
1.	***Casearia tomentosa* Roxb.**/ Flacourtiaceae	+	+	+	+	+	+	30 ml of the boiled water with extract of the root bark is taken to treat diabetes.
2.	***Cassia fistula*Linn.** / Caesalpiniaceae	+	–	+	+	+	+	20 ml of the boiled water with extract of the flowers is taken twice a day regularly by diabetic patients to reduce the blood glucose level.
3.	***Catharanthus roseus*(L.) G. Don /** Apocynaceae	+	+	+	+	+	+	20 ml of the boiled water with extract of flowers is taken thrice a day for a period of one month to treat diabetes.
4.	***Coccinia grandis* (L.) Voigt /**Cucurbitaceae	+	+	+	+	+	+	Five fresh fruits are taken regularly to prevent diabetes.
5.	***Dillenia indica* Linn. /**Dilleniaceae	+	+	+	+	+	+	50 ml of the boiled water with extract of the leaf is taken in morning a day regularly to treat diabetes.
6.	***Dillenia pentagyna* Roxb. /**Dilleniaceae	+	+	+	+	+	+	Unripe fruits are taken like vegetable or two ripe fruits are taken regularly to treat diabetes.
7.	***Ficus benghalensis* Linn. /**Moraceae	+	+	+	+	+	–	100g of the dried stem bark and root bark are continuously boiled in 250 ml of water with 50 g of the dried stem bark of Ficus hispida Linn. The decoction obtained is taken once a dayfor a period of six weeks to treat diabetes.
8.	***Momordica charantia* Linn. /**Cucurbitaceae	+	+	+	+	+	+	One premature fruit is taken regularly to prevent diabetes.

(Keys: + = Present, – = Absent)



Conclusion

The selected eight plants are the source of many active compounds and secondary metabolites, *i.e.*, alkaloids, flavonoids, carbohydrates, proteins, steroids, tannins and saponins. Medicinal plants play a vital role in preventing various diseases. which may be attributed to presence above mentioned compounds. Phytochemical screening of these plants may be helpful in developing specialized drugs with enhance efficiency. We propose that phytochemical properties of eight plants found in terai region of Uttar Pradesh, identified in our study will be helpful in the copping different diseases of this particular region.

Acknowledgement

The authors are grateful to the ethnic informants without whose active co-operation, the work could not be accomplished. Authors are also thankful to Dr. Anand Prakash (Ethnobotany and Ecology Division, NBRI- Lucknow) for his support during conducting the study.

Conflict of interest

We declare that we have no conflict of interest.

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