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A phytochemical analysis of medicinal plants of Dang District

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Abstract

Alkaloids, tannins, saponins, steroid, terpenoids, flavonoids, phenolic compounds, and glycosides distribution in five medicinal plants belonging to different families were assessed and compared in different medicinal plants that were collected from Dang district of Nepal. Qualitative analysis was carried out for the conformity of the presence of phytochemical compounds. The technique used for compound analysis was extraction method. Five plants taken for compound analysis were namely *Moringa olefera* (Moringaceae family), *Aegle marmelos* (Rutaceae family), *Azadirachta indica* (Meliaceae family), *Bauhinia variegata* (Fabaceae family), and *Tamarindus indica* (Fabaceae family).

Keywords: Medicinal plants; Phytochemical analysis; Alkaloids; Tannins; Saponins; Flavonoids

1. Introduction

Plants are integral part of nature and are important sources of medicine. Plants contain natural substance that can promote health. In previous time the plants were only the means of medication. The uses of plants as a medicine dates back to thousands of years from Ayurveda and it has the same value today as well. Nepal is also rich in plants and medicinal plants and the medicinal plants are used in various ailments.

Phytochemicals are naturally occurring chemical compounds in plants [1]. Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources and many of which based on their use in traditional medicine. The medicinal value of plants depends in some chemicals present in them. Among the 7,000 species of medicinal plants recognized so far all over the world, more than 900 types of precious medicinal plants are said to be found in Nepal [2]. The use of plants and plant products as medicines could be traced as far back as the beginning of human civilization. The earliest mention of medicinal use of plants in Hindu culture is founds in "Rigveda", which is said to have been written between 4500 - 1600 B.C. and is supposed to be the oldest repository of human knowledge. It is Ayurveda, the foundation of medicinal science of Hindu culture, in its eight division deals with specific properties of drugs and various aspects of science of life and the art of healing [3]. Medicinal plants contain some organic compounds which produce definite physiological action in human body and these bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids [4]. Many of these indigenous medicinal plants are used as spices and food plants. Medicinal plants are generally used in traditional medicine for the treatment of many ailments, [5].

According to World Health Organization (WHO), traditional medicine is estimated to be used by 80% of the population of most developing countries. These plant-based medicines are used for primary health care needs [6]. Although plants are unique in their activities, it has also been found that a particular plant may be used by different tribes or countries for different ailments. This shows that plants possess a wide range of healing powers which are attributed to their chemical composition. Despite the wealth of human experience and folklore concerning the medicinal uses of plants, proper scientific investigation has only been applied to a small fraction of the world's plants [7]. Plants generally have varied chemical compositions depending upon species. A good number of plants are known to be of economic and medicinal value. Those that are of medicinal value are often used as herbal remedy for the restoration and maintenance of good health. Some herbs have been considered as drugs and therefore generally safe and effective [8].

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2. Materials and Methods

2.1 Plant materials

Various medicinal plants are available in Nepal. Topographically all of the development zones and districts are quite rich in having the beneficial plants. For this research work Dang district was selected as a study area. Though

numerous medicinal plants are found in Dang but only 5 important plants were selected for the experimental analysis. The selected plant with their local name, scientific name (botanical name), plant family, the parts used for experiment and their medicinal uses is given below in a tabulated form.

Table 1: Ethno botanical information of selected medicinal plant species for phytochemical analysis in Dang District area of Nepal.

S. No	Local Name	Botanical Name	Family	Part used	Uses
1	Sajiwon	Moringa olefera	Moringaceae	Leaf	diabetes, heart, liver, respiratory, digestive, skin, diseases, anemia, arthritis
2	Bel	Aegal marmelos	Rutaceae	Leaf	jaundice, asthma, inflammations, bronchitis, snakebite, abdominal discomfort, epilepsy, leprosy, myalgia, smallpox, spermatorrhoea, leucoderma, eye, mental, nausea, ulcer disorders, infections
	Neem	Azadirachta indica	Meliaceae	Leaf	Antipyretic, Gastrointestinal, respiratory disorders, leprosy
4	Koiralo	Bauhinia veriegata	Fabaceae	Leaf	Diabetes, pneumonia
5	Imlichi	Tamarindus indica	Fabaceae	Leaf	Antipyretic, Carminative

2.2 Chemicals

Ethanol, Distill water, Aqueous HCL, Methanol, Chloroform, Concentrated Sulphuric acid, Ammonia solution, Picric acid, Acetic acid, Potassium mercuric iodide solution, Lead acetate solution

2.3 Sample collection

Plant were identified and authenticated then fresh and tender leaves of selected five medicinal plants, Moringa olefera (leaves), Aegal marmelos, Azadirachta indica, Bauhinia veriegata and Tamarindus indica were collected from different regions of Dang district of Nepal. The plants were used for the purpose of their phytochemical analysis. Plant species selected during present investigation are given in Table 1.

2.4 Preparation of plant extracts

The leaves of selected plants were collected, cleaned and air dried under shade. After drying, the leaves were then blended using a household electric blender into fine powder and transferred into airtight containers with proper labeling. This powder was analyzed for the phytochemicals present in it. Extraction of the plant parts was done with ethanol and water (1:1). The extract of the leaf, stem and root were obtained through the cold percolation method. The powdered plant material 50 gm was weighed and kept in a conical flask then soaked in 100 ml. of aqueous ethanol (1:1). The mouth of conical flask was covered with aluminum foil and kept for one week. Then the extract was taken by filtering the content. The extract was pooled and concentrated on a water bath by keeping the temperature 60 °C. The concentrated extract was kept in the desiccator for further evaporation of the solvent. These aqueous ethanol crude extracts of above mentioned plants were screened for the presence of various phytoconstituents such as steroids, alkaloids, glycosides, flavonoids, saponins, terpenoids, tannins, and phenolic compounds. The crude extracts were subjected to phytochemical analysis using standard methods.

2.5 Qualitative phytochemical analysis

The crude plant extract was obtained. The extracts were used for the preliminary phytochemical analysis to identify the various chemical constituents. All the tests were performed in triplicate mode so as to make more and more accurate. The standard procedures taken for analysis were ^[9, 10], and ^[11] as given below based on the results obtained in the qualitative phytochemical analysis.

- 1. Test for Alkaloids: Mayer's Reagent:** In 1ml. of the plant extract, 2ml. of Mayer's reagent (potassium mercuric iodide solution) was added. Appearance of dull white precipitate indicated the presence of alkaloids.
- 2. Test for Flavonoids:** (A) In 1ml. of plant extract, 1ml. of neutral ferric chloride was added. The formation of brown color confirmed the presence of flavonoids. (B) Crude extract was mixed with 2ml. of 2% solution of NaOH. An intense yellow color was formed which turned colorless on addition of few drops of diluted acid which indicated the presence of flavonoids.
- 3. Keller-Kilani test:** A mixture of Acetic acid glacial (2 ml.) with 2 drops of 2% FeCl₃ solution was added to the plant extract and H₂SO₄ concentrated. A brown ring at the interface indicated the presence of cardiac glycosides.
- 4. Test for Steroids: Lieberman-Burchard's Test:** The plant extract was dissolved in 2ml. of chloroform to which 10 drops of acetic acid and five drops of concentrated sulphuric acid were added and mixed. The change of red color through blue to green indicated the presence of steroids.
- 5. Test for Triterpenoids: Salkowski Test:** 5 ml. of each extract was mixed in 2ml of chloroform and concentrated sulphuric acid (3ml.) and it was carefully added to form a layer. A reddish brown precipitate of the interface indicated the presence of triterpenoids.
- 6. Test for Phenols:** In 1ml. of each extract, lead acetate solution was added and the precipitate formation indicated the presence of phenolic compounds.
- 7. Test for phenols and tannins:** Crude extract was mixed with 2ml. of 2% solution of FeCl₃. A blue-green or black coloration indicated the presence of phenols and tannins.
- 8. 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.

2.6 Data Analysis

The change of color was observed when the test reagent was added to the prepared sample for the phytochemical test. The result was recorded as sign plus (+) for presence and sign minus (-) for absence of organic compounds depending on the outcome of the test. All experiments were done in triplicate model so as to make it more and more accurate.

3. Results

The phytochemical test of the crude ethanol extracts of five medicinal plants (Moringa olefera, Aegal marmelos,

Azadirachta indica, Bauhinia veriegata and Tamarindus indica) was carried out. Among various available phytochemicals, this experiment was confined to observe the presence of eight important phytochemicals namely alkaloids, tannins, saponins, glycosides, flavonoids, steroids, phenols and triterpenoids. Phytochemical analysis of Moringa olefera showed the presence of alkaloids, tannins, saponins and steroids. Aegle marmelos also showed the presence of four phytochemicals which are alkaloids, tannins, flavonoids and steroids. Azadirachta indica showed the presence of six

phytochemicals that are alkaloids, tannins, glycosides, flavonoids, steroids and phenols. Bauhinia veriegata showed the presence of seven ^[7] phytochemicals such as alkaloids, tannins, saponins, glycosides, flavonoids, phenols and triterpenoids. And Tamarindus indica also showed the presence seven phytochemicals alkaloids, tannins, glycosides, flavonoids, steroids, phenols and triterpenoids. Table given below illustrates the presence and absence of eight ^[8] phytochemical compounds in five ^[5] medicinal plants:

Table 2: Laboratory analysis chart

Plants	Alkaloids	Tannins	Saponins	Glycosides	Flavonoids	Steroids	Phenols	Triterpenoids
Moringa olefera	+	+	+	-	-	-	-	-
Aegle marmelos	+	+	+	-	+	+	-	+
Azadirachta indica	+	+	+	+	+	-	+	-
Bauhinia veriegata	+	+	+	+	+	-	+	+
Tamarindus indica	+	+	-	+	+	+	+	+

Note: (+) indicates presence of organic compound
(-) indicates absence of organic compound

4. Discussion

1. Alkaloids and tannins were found present in all five plants species. This shows that alkaloids and tannins are dominantly available. Out of five analyzed plants saponins and flavonoids found in four plants. Flavonoid was absent in Moringa olefera, saponin is absent in Tamarindus indica.
2. Steroids are found only in two species out of five. It shows its scantiness. It reveals that flavonoids and steroids are also frequently found compounds in most of the medicinal plants.
3. Phenol was found in three medicinal plants Azadirachta indica, Bauhinia veriegata and Tamarindus indica and Triterpenoids are found in Aegle marmelos, Bauhinia veriegata and Tamarindus indica. This indicates that these two plants Bauhinia veriegata and Tamarindus indica are good source of both phenol and triterpenoid.

4.1 Summary

Crude plant extracts have both toxic and beneficial effect. Alkaloids are heterogeneous group compounds which contain one or more nitrogen atom in acyclic system. These are widely used in medicinal purposes which have positive and negative effects even to human beings ^[12]. Most of the plants have alkaloids in different organs with different chemical configurations ^[13] (Harborne, 1984). Alkaloids are reported to have analgesic, anti-inflammatory and adaptogenic activities which help to alleviate pains, developed resistance against diseases and endurance against stress ^[14]. The alkaloids, tannins and flavonoids are known to have curative activity against several pathogens and therefore could be used for the treatment of various illnesses ^[15, 16].

Glycosides are known to lower the blood pressure according to many reports ^[17]. Phenolic compound possess biological properties such as apoptosis, anti-aging, anti-carcinogen, anti-inflammation, anti-atherosclerosis, cardiovascular protection and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities ^[18]. The plant extracts were also revealed to contain saponins which are known to produce inhibitory effect on inflammation ^[19]. Steroids have been reported to have antibacterial properties ^[20] and they are very important compounds especially due to their relationship with compounds such as sex hormones ^[21]. The growth of many fungi, yeasts, bacteria and viruses can be

inhibited by tannins ^[22]. Terpenoids and tannins are attributed for analgesic and anti-inflammatory activities. Although, the absence of certain phytochemicals in one sample and its presence in the other can be safely attributed to the various physiological and biosynthetic reactions taking place inside the plant, the effect of the environment should not be neglected, as the environment always modify the things. The preliminary phytochemical tests are therefore significant and helpful in finding chemical constituents in the plant material that may lead to their quantitative estimation and also in locating the source of pharmacologically active chemical compounds ^[23].

Phenols are reported antitumor agents and to exhibit antiviral and antimicrobial activities ^[24] hypotensive effect ^[25] and antioxidant properties ^[26]

The results obtained in this study thus suggest the identified phytochemical compounds may be the bioactive constituents and these plants are evidencing to be an increasingly valuable reservoir of biologically active ingredients of substantial medicinal value.

5. Conclusion

phytochemical studies were performed it was found that the ethanolic extracts of the considered plants contained many bioactive chemical constituents including alkaloids, glycosides, terpenoids, saponins, tannins, steroids, flavonoids and phenols. Several earlier studies also confirmed that the identified phytochemicals to be bioactive and these phytochemicals contribute medicinal as well as physiological properties to the plants studied in the treatment of diverse ailments.

5.1 Recommendations

The traditional medicine practice is recommended strongly for these plants as well as it is suggested that further work should be carried out to isolate, purify, and characterize the active constituents responsible for the activity of these plants. Also additional work is encouraged to see whether these plants have said health benefits, especially as anti-cancer drugs, and elucidate the possible mechanism of action of these extracts. From the above result it can be said that all the five experimented plants species are rich in important phytochemical compounds that are highly useful for

medicinal uses so plantations, protection and conservation of these medicinal plants seem essential.

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