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Preliminary Phytochemical Screening of some compounds from plant stem bark extracts of *Tabernaemontana divaricata* Linn. used by Bodo Community at Kokrajhar District, Assam , India

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ABSTRACT

The phytochemical analysis of stem bark extracts in petroleum ether, chloroform, methanol and aqueous extracts of indigenous medicinally important plants of Tabernaemontana divaricata were investigated. The phytochemicals analysis reveals the presence of alkaloids, flavonoids, glycosides, lignins, saponins, tannins, carbohydrates, amino acids and quinines. This research supports the local use of the stem bark extract of the plant Tabernaemontana divaricata for treatment of fever. The present study provides evidence that solvent extract of Tabernaemontana divaricata contains medicinally important bioactive compounds and this justifies the use of plant species as traditional medicine for treatment of various diseases.

Key words*: Tabernaemontana divaricata* Linn., indigenous, stem bark extract, Phytochemical.

INTRODUCTION

Medicinal plants have been used as the remedies of human diseases for century because they contain components of therapeutic value. Some of them are also used for prophylactic purposes. An increasing interest in herbal remedies has been observed in several parts of the world and many of the herbal remedies have been incorporated into orthodox medicinal plant practice. Diseases such as malaria, epilepsy, infantile convulsion, diarrhea, dysentery, fungal and bacterial infections have been managed by traditionally using of medicinal plant (Sofowora, 1996). Medicinal herb is considered to be a chemical factory as it contains multitude of chemical compounds like alkaloids, glycosides, saponins, resins, oleoresins, sesquiterpene, lactones and oils (essential and fixed) (Singh, 2005). India has one of the oldest, richest and most diverse cultural traditions associated with the use of medicinal plants.

According to an estimate, 120 or so plant based drugs prescribed for use through the world come from just 95 plant species (Lewington, 1990).Natural antimicrobials can be derived from plants, animal tissues and microorganisms (Zaika,1975). The shortcomings of the drugs available today propel the discovery of new pharmacotherapeutic agents from medicinal plant research (Cordell, 1993). The amount of phytochemical substances varies considerably from species to species and even from plant to plant, depending on the age and various ecological and climatic factors (Baquar,1989). Plants have limitless ability to synthesize aromatic substances, mostly phenols or their oxygen-substituted derivatives (Geissman,1963). Most of the natural products are secondary metabolites and about 12,000 of such products have been isolated so far. These products serve as plant defence mechanisms against predation by microorganisms, insects and herbivores (Fransworth and Morris,1976). Today there is growing interest in chemical composition of plant based medicines. Several bioactive constituents have been isolated and studied for pharmacological activity. During the last two decades, the pharmaceutical industry has made massive investment in pharmacological and chemical researches all over the world in an effort to discover much more potent drugs, rather, a few new drugs. Plants have successfully passed the tests of commercial screenings.

Tabernaemontana divaricata Linn. (Apocynaceae) (Synonyms: *Ervatamia coronaria* and *Tabernaemontana coronaria*), commonly known as Wax flower, Crepe flower, Crepe jasmine in India, Kathana in Assam and Dowdwi phool in Bodo language is a glabrous, evergreen shrub 1.8-2.4 m in height with silvery grey bark and milky latex. It bears attractive, white coloured fragrant flowers. The plant flowers throughout the year in the climatic condition of Assam but it blooms in autumn season heavily. The leaves are shiny and deep green in colour. *Tabernaemontana divaricata* is found in Tropical Asia, Australia and Polynesia. In India, it occurs in upper Gangetic plain, Garhwal, East Bengal, Khasia Hills, Assam, Burma, hills of Vishakapatnam. It is cultivated as an ornamental plant, grows wild in hedges and shady forests (Kirthikar and Basu, 1998; National Institute of Science Communication, Council of Scientific and Industrial Research, 2000).

The phytochemistry and a number of chemical constituents such as alkaloids, terepenoids, steroids, flavonoids, pheny propanoids, phenolic acids and enzymes from the leaves, stems, and roots have been reported previously (Arambewela and Ranatunge, 1991; Kam and Anuradha, 1995; Fulton *et al.*, 1994; Sierra, 1991). In traditional medicine *Tabernaemontana divaricata* is used to treat various diseases like epilepsy, abdominal tumours, eye infections, fractures, fever , headache, inflammation, mania, oedema , leprosy, diarrhea (Ghani, 2003). In the view of its traditional use for treatment of various diseases, the aim of the present study is to find out phytochemical active constituents of plants extracts of *Tabernaemontana divaricata*.

MATERIALS AND METHODS

Plant material collection and identification

The experimental plant sample (stem bark) *Tabernaemontana divaricata* Linn. was collected from the forest areas of Patgaon of Kokrajhar District, Assam, India with the help of local tribal people in the month of November, 2015. The plant material was identified by NEHU HERBARIU, Department of Botany, NEHU, Shillong, Meghalaya, India, where a voucher specimen has been deposited for future reference. After identification, the plant stem barks were collected and then cut into pieces and washed under running tap water to remove adhering dirt. Then stem barks were sun dried for 5 days. Dried plant material was powder using mixture grinder and stored in the air tight container for further use.

Preparation of Plant Extracts

Petroleum ether, Chloroform and Methanolic extract

About 100gm of powered material was taken separately in a clean, flat-bottomed glass container and soaked in 500ml of chloroform, petroleum ether and 90% methanol. The container with its contents was sealed and kept for a period of 7 days accompanying occasional shaking and stirring. The solution was filtered using Whatman filter paper (No.1). The filtrates were then evaporated under reduced pressure and dried using a rotary evaporator at 55°C. Dried extracts were stored in labelled sterile screw capped bottles at 5°C in the refrigerator, until when required for use.

Aqueous extract

Similarly, 50 g of the plant powdered material was soaked in 400 ml of distilled water. This was heated to boil using hot plate. The mixture was stirred at regular intervals (3-5 min) for one hour after which it was filtered with No. 1 Whatman filter paper. The filtrate (aqueous extract) thus obtained was concentrated in a hot water bath at 80° C for 5 h. The filtered extract was then refrigerated at 5°C until required for use.

Chemicals and reagents

All the chemicals and reagents were of analytical grade and were purchased from Merck Chemicals Pvt. Ltd., Mumbai and Nice Chemicals Pvt. Ltd., Kerala.

Preliminary Phytochemical screening of Secondary Metabolites

The petroleum ether, chloroform, methanol and aqueous extracts were subjected to preliminary phytochemical screening such as Alkaloids (Iodine, Wagner's and Mayer's test), Flavonoids (Pew's, Shinoda and NaOH tests), Glycosides (Keller-Kiliani, Glycosides, Conc.H2SO_4, and Molisch's tests), Phenols (Ellagic acid test), Lignin (Lignin and Lebat tests), Saponins (Foam test), Sterols (Salkowski tests), Tannins (Ferric chloride and Lead acetate tests), Carbohydrates (Molisch's, Barfoed's and Seliwanoffs tests), Protiens (Biuret test), Amino acids (Millon's tests) and Quinones test were carried out.

Preliminary Screening of Phytochemical Test

Phytochemical Screening

The extract obtained was subjected to Preliminary Phytochemical screening.

Test of Alkaloids

Iodine Test: To 3 ml test solution, a few drops of dilute iodine solution were added. Blue colour appears; it disappears on boiling and reappears on cooling (Khandewal, 2008).

Wagner's Test: To 2-3 ml extract with few drops Wagner's reagent were added. Formation of reddish brown precipitate indicates the presence of alkaloids (Kokate *et al;* 2001).

Mayer's Test: To 1 ml of test solution or filtrate was added a drop or two of the Mayer's reagent along the sides of the test tube. A white or creamy precipitate confirmed the test as positive (Harborne, 1998; Kokate, 2005).

Test for Flavonoids

Pew's Tests: To 2-3 ml extract, zinc powder was added in a test tube, followed by dropwise addition of concentrate HCl. Formation of purple red or cherry colour indicates the presence of flavonoids (Peach and Tracey, 1956).

Shinoda Tests: To 2-3 ml extract, few fragments of magnesium metal were added in a test tube, followed by dropwise addition of concentrate HCl. Formation of magenta colour indicated the presence of flavonoids (Kokate *et al.,* 2001).

NaOH Tests: To 2-3 ml of extract, few drops of sodium hydroxide solution were added in a test tube. Formation of intense yellow colour that became colourless on addition of few drops of dilute HCl indicated the presence of flavonoids (Khandewal, 2008).

Test for Glycosides

Keller-Kiliani Test: To 2 ml extract, glacial acetic acid, one drop 5% $FeCl_3$ and conc. $H2SO_4$ were added. Reddish brown appears at junction of the two liquid layers and upper layer appears bluish green indicates the presence of glycosides (Kokate *et al.,* 2001)

Glycosides Test: To small amount of extract was mixed with 1 ml water and was shaken well. Then aqueous solution of NaOH was added. Yellow colour appeared that indicates the presence of glycosides (Treare and Evans, 1985)

Concentrate $H2SO_4$ Test: To 5ml extract, 2ml glacial acetic acid, one drop 5% $FeCl_3$ and conc. $H2SO_4$ were added. The appearance of brown ring indicates the presence of glycosides (Khandewal, 2008).

Molisch's Test: To 1ml of extract, 2 drops of Molisch's reagent was added in test tube and 2 ml of concentrate $H2SO_4$ was added carefully keeping the test tube slightly curved. Formation of violet ring at junction indicated the presence of glycosides (Kokate *et al.,* 2001)

Test for phenols

Ellagic Acid Test: The test solution was treated with few drops of 5% (w/v) glacial acetic acid and 5% (w/v) $NaNO_2$ solution. The solution turned muddy or niger brown precipitate occurred in the extract indicated the presence of phenols solution (Gibbs, 1974)

Test of Lignins

Lignin Test: To 2 ml of 2% (w/v) furfuraldehyde was added to the test solution, formation of red colour indicated the presence of lignin (Gibbs, 1974).

Labat Test: The test solution was mixed with gallic acid; it developed olive green colour indicating the positive reaction of lignins (Gibbs, 1974)

Test for Saponins

Foam Test: The extract was diluted with 20 ml of distilled water and it was shaken in a graduated cylinder for 15 minutes. A 1 cm. layer of foam indicated the foam of saponins (Kokate *et al.,* 2001)

Test for Sterols

Salkowski Test: To 2 ml of extract, 2 ml chloroform and 2 ml concentrated $H2SO_4$ was added and was shaken well. Chloroform layer appeared red and acid layer showed greenish yellow fluorescence indicated the presence of sterols (Kokate *et al.,* 2001)

Test for Tannins
Ferric chloride test: To 2 ml of test solution, a few drops of 5% ferric chloride solution was added. Formation of blue color indicated the presence of hydrolysable tannins ((Harborne, 1998; Kokate, 2005)

Lead acetate test: To 5 ml of extract, a few drops of 10 % lead acetate solution was added. Formation of yellow or red precipitate indicated the presence of tannins (Treare and Evans, 1985)

Test for carbohydrates
Molisch's Test: To 1 ml of test solution, a few drops of 1 % alpha-napthol and 2-3 ml concentrated sulfuric acid were added along the side of test tube. The reddish violet or purple ring formed at the junction of two liquids confirmed the test (Harborne, 1998; Kokate, 2005)

Barfoed's Test: To 2ml of reagent was added to 2 ml of test solution and was mixed and kept a in boiling water bath for 1 min. Red precipitate formed indicates the presence of monosaccharides (Harborne,1998; Kokate,2005)

Seliwanoffs Test: To 3 ml of Seliwanoffs reagent was added to 1 ml of the test sample and heated on a water bath for one minute. The formation of rose red color confirmed carbohydrates (Harborne, 1998; Kokate, 2005)

Test for Proteins
Biuret test: To 2 ml of the test solution, 5 drops of 1% copper sulphate solution and 2 ml of 10% NaOH was added and were mixed thoroughly. Formation of purple or violet color confirmed proteins (Harborne, 1998; Kokate, 2005)

Test for Amino acids
Millon's test: To 1 ml of test solution, 5 drops of millons reagent was added and heated on a water bath for 10 min, cooled and 1% sodium nitrite solution was added. Appearance of red color confirmed the test (Harborne, 1998; Kokate, 2005)

Test for Quinone
Extracts was treated with concentrated HCl appearance of green colouration indicates presence of Quinone (Khandelwal ,2000)

RESULTS AND DISCUSSION

The results of the phytochemical analysis of the stem bark extracts in various solvents has shown a remarkable variation in the presence the above studied phytochemical compounds in the studied taxa. The detailed investigations of phytochemicals in various solvents are shown in TABLE 1. The study reveals that the stem bark extracts of *Tabernaemontana divaricata* are showing maximum presence of alkaloids and carbohydrates in petroleum ether and aqueous extracts but are adequately present in methanol extacts, whereas alkaloids and carbohydrates are completely absent in Chloroform extracts. Flavonoids are highly present in methanol extracts but in aqueous extracts flavonoids are adequately present, whereas flavonoids are completely absent in petroleum ether and Chloroform extracts. In aqueous and methanol extracts, tannins are highly present but are completely absent in petroleum ether and Chloroform extracts. Saponins and amino acids on other hand are maximum in aqueous extracts, whereas saponins and amino acids are adequately present in methanolic extracts but are completely absent in petroleum ether and chloroform extracts . Glycosides are maximum in petroleum ether and Chloroform extracts, whereas glycosides are adequately present in aqueous extracts but are completely absent in methanol extracts. Lignins on other hand are maximum in methanol extracts but are adequately present in petroleum ether extracts, whereas lignins are completely absent in chloroform and aqueous extracts. In petroleum ether extracts, quinones are present strongly but are adequately present in methanol extracts whereas quinones are completely absent in chloroform and aqueous extracts. However, in all the extracts phenols, sterols and proteins are completely absent (Table 1).

Table 1. The Preliminary Phytochemical screening of the various Extract of stem bark of *Tabernaemontana divaricata* Linn.

Chemical Tests	Petroleum Ether	Chloroform	Methanol	Aqueous
1.Test for Alkaloids				
a) Iodine Test	-	-	-	-
b) Wagner's Test	++	-	-	+
c) Mayer's Test	-	-	+	++
2.Test for Flavonoids				
a) Pew's Test	-	-	-	-
b) Shinoda Test	-	-	+ +	+
c) NaOH Tests	-	-	-	-
3. Test for Glycosides				
a) Keller Kiliani Test	-	-	-	-
b) Glycosides Test	-	-	-	-
c) Cocentrate $H2SO_4$ Test	-	+ +	-	+
d) Molisch's Test	+ +	+	-	-
4. Test for Phenols				
a) Ellagic Acid Test	-	-	-	-
5. Test for Lignins				
a) Lignin Test	-	-	-	-
b) Labat Test	+	-	+ +	-
6. Test for Saponins				
a) Foam Test	-	-	+	+ +
7. Test for Sterols				
a) Salkowskis Test	-	-	-	-
8. Test for Tannins				
a) Ferric chloride Test	-	-	-	-
b) Lead Acetate Test	-	-	++	+ +
9.Test for Carbohydrates				
a) Molisch's Test	+ +	-	-	++
b) Barfoed's Test	-	-	+	-
c) Seliwanoff's Test	-	-	-	-
10) Test for Protiens				
a) Biuret Test	-	-	-	-
11) Test for Amino acids				
a) Millon's Test	-	-	+	+ +
13) Test for Quinone	+ +	-	+	-

++ = Strongly present, + = Slightly present and - = Absent

CONCLUSION

Thus, from the above study, the stem bark extracts of *Tabernaemontana divaricata* showed an abundant production of Phytochemicals as secondary metabolites and they can be used in the pharmaceutical industries for producing a potent drug against fevers. The studies result of this plant gives a basis of its use in traditional medicine to manage ailments and disorders. It also contains some biologically active constituents worthy of further investigations.

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